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THE BOTANICAL REVIEW

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FINE STRUCTURE IN DEGRADED, ANCIENT AND BURIED WOOD, AND OTHER FOSSILIZED PLANT DERIVATIVES

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Introduction

In recent decades various aspects of cell-wall structure in different groups of plants have attracted the attention of biophysicists and textile technologists, and the essentials of a botanical approach to the subject have been masterly presented in two excellent and rather recent monographs (Preston, 1953; Frey-Wyssling, 1953). In a separate review (Frey-Wyssling, 1952) the deformation of plant cell-walls, with special reference to their rheological properties, has been considered. Bailey was the first to stimulate interest in cell-wall structure among American botanists, and he reviewed the difficult situation in an excellent article (Bailey and Berkley, 1942) which has been followed by a few general reviews in recent textbooks (e.g., Meyer and Anderson, 1952; Esau, 1953).

The result of all these investigations has been a voluminous literature on various aspects of cell-wall structure, without, however, giving consideration to that structure as found in degraded, buried and ancient wood. Hence, a brief attempt is made in the first part of this paper to review some phases of cell-wall structure [1] which have received the interest of botanists only very recently.

Similarly, the structure of greatly altered vegetable materials, converted into economic fuels, has received wide attention from chemists, physicists and fuel technologists. As a result, the complex submicroscopic morphology of coal is now being approached from different angles, and some excellent accounts of fine structure

[1] "Structure" refers to the crystalline fine cellulose structure of the plant cell-wall, whereas "texture" is only the pattern of arrangement and distribution of the structural units.

in coal have appeared (Mahadevan, 1940; Horton, Randall and Aubrey, 1944; Proc. Ultra-fine structure of coals and cokes, B.C.U.R.A., 1944; Brown and Hirsch, 1955). But a review of available literature on the fine structure in fossil fuels, with special reference to the probable original structural patterns in their woody mother substance, which interests a botanist, is lacking. Moreover, other fossilized plant derivatives also deserve attention. The second part of this review is, therefore, essentially a botanical approach to the study of fine structure in fossilized plant derivatives. Obviously, frequent references to and descriptions of some physical and chemical aspects of coal structure, based on structural units which are not the same as existing in plants, must also be considered.

I. Fine Structure in Degraded, Ancient and Buried Wood

It is desirable to briefly describe the nature and structure of the cell-wall of normal wood fibres and of tracheids as a basis of comparison with corresponding degraded, ancient and buried structures. In normal fibres and tracheids cellulose is the basic constituent of the cell-wall. Among other wall constituents which are variously associated with the skeletal cellulose are lignin, hemicellulose and pectin, but quantitatively cellulose often prevails with more than 50%. Long chain molecules of anhydroglucopyranose residues go into the building up of cellulose. These molecules are organized into micelles which form a crystal lattice. A micelle is at least 600 Å long and 50–60 Å wide. Between these micelles is the non-oriented cellulose. Varying amount of amorphous cellulose, over and above the crystalline cellulose, may also be in the cell-wall. The anisotropy of the wall depends on the orientation, angular dispersion, etc., of the crystalline chains, but the precise value of any property in any particular direction in a piece of cellulose will depend to a large extent on the amount and degree of alignment in the non-crystalline fraction (Preston, 1952).

It has been stated that about 25 crystalline cellulose micellar strands are held together by a non-crystalline fraction. They aggregate to form a microfibril 200–300 Å in diameter (Frey-Wyssling, 1952) which is revealed by the electron microscope. Broadly speaking, the microfibrils are laid down parallel in the secondary walls in fibres and tracheids, and are dispersed in primary walls.

A typical normal wood fibre or tracheid usually consists of a

primary wall, formed in the initial or cambial stage of development, and a three-layered secondary wall. Between any two adjacent fibres or tracheids lies the so-called middle lamella, or the intercellular substance. In a cross section of a fibre or tracheid, the inner and the outer secondary wall layers exhibit pronounced anisotropy in contrast to the isotropic central layers. The primary wall is rather weakly birefringent. In longitudinal section, however, the central layers show maximum anisotropy. Such optical heterogeneity has been variously explained by different authors. It has been stated that the cellulose micellar chains in the outer and the inner secondary wall layers are usually in flatter spirals than those in the central secondary wall layers.

Preston (1934, 1946), working on conifer tracheids, however, maintains that the optical heterogeneity, to a considerable extent, is due to a pronounced difference in angular dispersion between the layers, the cellulose chain directions with respect to the longitudinal axes of the wood fibres or tracheids remaining constant. More recently Wardrop and Preston (1947) concluded that both the orientation of the cellulose and micellar spirals, and the angular dispersion about them, are responsible for the optical heterogeneity referred to. Such a conclusion is based on critical examination of birefringence of the cell-wall layers in sections cut at various suitably tilted angles and on subsequent X-ray examinations.

Secondary wall layers are cleavable into microscopically visible fibrils which lie parallel. These fibrils also run parallel to the striations or slit-like pit orifices on the cell-wall, and they are said to correspond to the directions of micellar slope. Fibrillization may not substantially alter the orientation of cellulose, " since the m.e.p. did not alter by more than 5° during the crushing process and treatment with alkali " (Wardrop and Dadswell, 1948).

Linear arrangements of structural units, whether macroscopic, microscopic or submicroscopic, seem to be a general principle in the skeletal substance of the cell-wall. " Although the diameter of a cellulose molecule is a million times smaller than that of a fibre strand, the architecture is fundamentally the same in both " (Frey-Wyssling, 1955).

Baily and Berkley (1942) believe that use of the polarising microscope and of X-ray diffraction patterns alone may lead to serious misconceptions regarding the orientation of cellulose in cell-wall layers unless the morphological, histological, chemical and

other variables in plant materials are accurately examined and accounted for. So the available information on the microscopic cell-wall architecture, etc., of degraded, ancient and buried materials has also been considered in terms of its submicroscopic structure and/or texture. Unfortunately, the low and at the same time degraded cellulose contents in some ancient fibres and tracheids do not always retain much of the original structures for such studies. Such ancient samples, however, sometimes provide valuable information regarding fossilization.

CELL-WALL ARCHITECTURE IN DEGRADED RECENT WOOD

There has been systematic work to find out the mechanism of microbial breakdown of cellulose and the factors affecting the susceptibility of cellulose to microbial attack (Siu and Reese, 1953), but what happens to the cell-wall architecture in wood after the attack is no less interesting. Similarly, the nature and arrangement pattern of cellulose and of the other important cell-wall constituents in buried and ancient wood reveal interesting phases of variable cellulose disorientation.

Most of the principal organisms attacking wood belong to the families of higher fungi, namely, Agaricaceae, Polyporaceae, Thelephoraceae, etc. These organisms mainly attack either cellulose or lignin, but often both at some stages of decay.

When cellulose is attacked, the structural framework of the cell walls gradually collapses. Schulze et al. (1938) have found by the X-ray diffraction method that the action of certain cellulose-destroying fungi (e.g., *Coniophora cerebella, Poria vaporaria* and *Merulius lacrimans*) in wood results in gradual disappearance of the submicroscopic fine cellulose orientation pattern. In some cases the cell-wall outlines are maintained before complete disintegration by finely deposited lignin residues. Using a Spierer contrast lens, Thiessen (1932) also found that in decaying wood the lignin reveals the general line up of lost cellulose. The cell-wall residues are composed of fine microscopic super-micelles in perfect alignment, showing a considerable degree of preferred orientation. This phenomenon has since been observed also by the present reviewer (Sen, 1955, unpub. a).

Spierer pictures of ancient wood exhibit preferred orientation of the super-micelles with reference to the long axis of the cell in the lignin-rich organisation of the cell-wall. A similar pattern was

earlier constructed for lignin orientation by Lange (1944, 1945) who, by measuring the dichroism of spruce lignin in ultra-violet light, found further that the native lignin in the middle lamella as well as in the cell wall shows self dichroism. This means that the lignin must be oriented or deposited in a preferred direction, at least to some extent. Therefore, these preferred patterns of lignin and the usual patterns of cellulose are generally likely to be corroborative; the one retains the general form of the other when the latter is absent. Spierer pictures, even though produced solely by lignin, offer testimony regarding the original orientation pattern of cellulose. Such is the nature of the fine cell-wall architecture in heavily infected decaying wood wherein cellulose has been mostly destroyed.

When the action of a fungus (e.g., *Trametes pini*) involves the destruction chiefly of lignin, the submicroscopic architecture of the cellulose micelles is excellently preserved, as revealed in X-ray diffraction photographs (Schulze et al., 1938). Under the Spierer lens the microscopic super-micelles in cellulose-rich cell walls are also regularly oriented. But when cellulose is destroyed, its orientation pattern is left on the persistent lignin. Therefore, lignin preserves only a textural pattern in the absence of cellulose.

RELATION BETWEEN FUNGAL ACTION AND CELLULOSE ORIENTATION

Bailey and Vestal (1937) have established correlations between fibrillar orientations and planes of enzymatic hydrolysis of certain ubiquitous fungi. In the secondary walls of tracheary cells the fungal enzyme hydroylses along two sets of predetermined planes. One such plane of hydrolysis is oriented parallel to the long axis of the fibrils of cellulose. The hyphae and the decay cavities are oriented with their long axis parallel to the long axis of the fibrils. In the " inner and outer layers of the secondary walls of tracheids, they are oriented transversely or in helices of low pitch, whereas in the central layer of such walls, they are oriented longitudinally or in helices of comparatively steep pitch with respect to the long axis of the tracheids ". The decay cavities on such tracheids and fibres, the inclination of which can be derived from the cellulose chain lattice as shown by Frey-Wyssling and Mühlethaler (1951), prove that the crystal lattice of adjacent micellar strands, and even of neighbouring microfibrils, must have the same orientation as their " a " and " c " axes (Frey-Wyssling, 1938).

The present reviewer (Sen, 1948), however, could not establish a constant correlation between the orientation of fibrils and the plane of enzyme action in *Lenzites striata*-infected sapwood tracheids of chir (*Pinus longifolia*). The fibrils of samples examined were in helices of low pitch in the outer (and also possibly in the inner) layers, and were very steep to almost parallel with reference to the long axis of the cells in the central layers as usual.

The present reviewer, in collaboration with Banerjee (1950), pursued studies of similar but only slightly infected materials with reference to control by employing the X-ray diffraction method. It has been found that the patterns, shape and size of the spots of intensity are not the same in the photographs of sound and infected materials. In sound samples the spots from the 101 and $10\bar{1}$ planes are diffuse and tend to merge into one interference ring, and they are drawn into a long diffuse arc along the Debye-Scherrer ring. Similarly, 002 interference is drawn into a long arc. But in infected samples the corresponding spots are sharp, and the other spots on the layer ring are also distinct. Consequently, the degree of disorientation of cellulose crystallites from the tracheal axis is much greater in sound chir wood than in the contiguous slightly infected portion. This shows the effect of enzymatic hydrolysis on the cellulose pattern, i.e., the cell wall architecture.

Probably the greater micellar orientation referred to as occurring in the sample slightly infected by a brown rot fungus, is related to longitudinal shrinkage of the tracheids [2]. The crystallinity may possibly be increased by removal of the disordered cellulosic fraction and other non-cellulosic substances by the enzymatic action of the fungus. Anyway it is difficult at this stage to conclude finally as to the mechanism that produces the results referred to. There may be some other factors involved in the process.

CELL-WALL ARCHITECTURE IN ANCIENT AND BURIED WOOD

GENERAL CHEMICAL NATURE OF THE CELL-WALL AS RELATED TO ITS STRUCTURE IN ANCIENT AND BURIED WOOD. The amount of cellulose in visibly degraded ancient and buried wood gradually decreases, as a rule, and this results in an apparent increase in lignin

[2] I have since been advised by Dr. C. A. Richards, Senior Pathologist, Forest Products Lab., U. S. Dept. of Agriculture, that brown rot fungi produce shrinkage of the tracheids in all directions, but possibly more in the longitudinal direction than otherwise.

content. Complete loss of cellulose may be considerably delayed in some cases (Mitchell and Ritter, 1934; Jurasky, 1938; Barghoorn and Spackman, 1950; Barghoorn, 1952). Lignin, on the contrary, decomposes very slowly and is far more resistant to decay than any of the cell-wall polysaccharides. The residual cellulose has usually been found degraded, as is evident from comparative viscosity and copper number data (Barghoorn, 1949b; Sen, unpub. a). It has been suggested by Jurasky (1938) and Barghoorn and Spackman (1950) that the extent of loss of cellulose is fundamentally an index to the degree of chemical and physical degradation. Such is the nature of cell-wall composition in ancient and buried wood. This aspect of the cell-wall is now being reviewed (Sen and Basak, unpub.).

All these facts are important in the present article in as much as they variably preserve in some way the cell-wall architecture in degraded ancient wood. Sometimes all cell structures are destroyed by excessive degradation. The decay usually proceeds from the surface of the wood towards the centre.

In some cases discussed in this article, the original wall constituents are so excellently preserved that the details of microscopic cell-wall morphology are as well revealed as in the cell-wall of recent and normal wood. Obviously, preservation of the structure and/or texture of the cell-wall depends entirely on the chemical nature of the wall constituents.

FIBRILLAR ORIENTATION IN ANCIENT AND BURIED FIBRES AND TRACHEIDS. The present reviewer (Sen, 1955, unpub. a) has found that in tracheids and fibres of visibly decomposed ancient and buried wood, which usually lack unaltered cellulose, the molecular structural pattern has been more or less destroyed. In such cases the cell-wall is not ordinarily cleavable into characteristic fibrils, since the fundamental structural units have been greatly altered. The lignin retains the original line-up of cellulose after it is destroyed, i.e., the arrangement of previous structural units is left preserved in lignin in the form of a texture. Drying of such lignin-rich tracheids and fibres produces rough cleavages on the wall along certain planes which usually correspond to the orientation of lost cellulose. Unfortunately, in the visibly decomposed ancient wood of *Picea excelsa* and *Heritiera fomes* the degree of orientation of such textural units (corresponding to the original

fibrils and, therefore, to the micelles) can not be measured because of irregularities in portions which have been almost completely destroyed. In the apparently sound core of *Picea excelsa* and two ancient hardwood species, namely, *Bischofia javanica* and *Shorea robusta,* wherein a good amount of unaltered cellulose is present, the cell walls are characteristically cleavable into fibrils. The angles of such fibrillar spiral slopes can be determined with accuracy.

OPTICAL PROPERTIES OF THE SECONDARY CELL-WALL LAYERS IN ANCIENT AND BURIED WOOD. Jahn and Harlow (1942) examined the cell-walls of ancient beech wood between the crossed nicols of a polarizing microscope. For the most part the cell-walls failed to transmit polarized light, an indication, substantiated by other reliable evidence, that the cellulose had been largely removed or altered. In scattered patches the outer layers of the secondary walls were birefringent. But whether this irregular birefringence was due to anisotropic cellulose or to some imbibition liquid has not been explained. The presence of form birefringence has actually been observed in the ancient buried wood of *Heritiera fomes* (Sen, 1955, unpub. a). So, apparently birefringence may not always be indicative of any truly regular micellar orientation.

Bailey and Barghoorn (1942), Barghoorn (1949b) and Barghoorn and Spackman (1950) also noted that the cell-wall layers of ancient wood samples sometimes exhibit true birefringence of clearly variable intensities which may be strong or feeble or even scarcely detectable. " In almost all cases, however, the tenuous outer layers of the secondary wall retained their form and their capacity for feeble birefringence ". Such birefringence may be greatly accelerated by delignification (Barghoorn, 1949b). These authors, however, did not mention anything about the possibility of the determination of major extinction position or birefringence in the different cell-wall layers for ascertaining more precisely the angle of micellar slopes in them.

The present reviewer (Sen, 1955, unpub. a) has experienced great difficulty in interpreting the optical nature of his visibly degraded materials of ancient tracheids and fibres in view of the unsatisfactory birefringent measurements and m.e.p. determinations. The cell-wall layers are thus optically irregular. Similarly, pieces of dicotyledonous wood from the Boylston Street Fishweir peat frequently show a mosaic of intensely birefringent cells scattered

among others that are feebly anisotropic or isotropic. This clearly shows that the degradation of the cellulose has progressed very irregularly, even in different parts of the same tissue (Bailey and Barghoorn, 1942).

However, the cell-wall layers in the ancient wood of *Pinus sylvestris,* apparently sound core of *Picea excelsa* and two well preserved hardwood species (*Bischofia javanica* and *Shorea robusta*) show characteristic optical heterogeneity as in their normal and recent representatives (Sen, unpub. a; Sen and Basak, 1955). This may indicate the differences in micellar orientation in the different wall layers as applicable to normal ones. These optical phenomena have been subsequently confirmed by X-ray diffraction studies. Mitchell and Ritter (1934) have also found that the undecomposed fibres of some Miocene conifers manifest preferred orientation of their cellulosic structures when viewed between crossed nicols with corresponding axes arranged at 90 degrees to one another.

Chemical analysis of all these visibly decomposed and relatively well preserved ancient and buried wood samples has shown variably preserved cellulose in them, quite consistent with optical and other data. Thus the fine structure of the cell wall in visibly degraded, ancient and buried wood has usually been found strongly destroyed, whereas the wonderful submicroscopic architecture in relatively well preserved samples is retained, often with great perfection.

SPIERER DARKFIELD IMAGES OF ANCIENT AND BURIED WOOD STRUCTURE. It has been demonstrated by Spierer (1930), Thiessen (1932) and others that the details of microscopic cell-wall architecture may be brought out with strong contrast in darkfield by Spierer oil immersion lens. The architectural units of the organic fibres, etc., thus revealed have since been designated as "supermicelles" (Seifriz, 1936a, b). The super-micelles may also be regarded as micelles in the strict sense because the cellulose micelles are sometimes as long as 1.7 μ, and thus they are comparable in size to super-micelles (1.5 μ to 2 μ) (Seifriz, 1936a). According to Thiessen (1932), these super-micelles are characteristically arranged in cotton, ramie and wood fibres, and verify the structure postulated by X-ray study. Spierer pictures thus appear to be faithful images of the finer details in fibres, etc.

The present reviewer (Sen, 1955, unpub. a) has noted that the super-micelles in ancient wood are arranged in various degrees of preferred orientation. The presence of irregular patches of super-micelles results from strain.

It has also been found that loss of cellulose and enrichment of lignin in wood produce better super-micellar pictures (Thiessen, 1932; Sen, 1955, unpub. a). So it is chiefly lignin that preserves the original architectural plan of wood when the cellulosic structural framework is gradually destroyed.

X-RAY ANALYSIS OF ANCIENT TRACHEIDS AND FIBRES. X-ray examination of ancient fibres was first made by Jahn and Harlow (1942). They found the cellulosic substance of ancient beech in a non-oriented arrangement with reference to the fibre axis. The 002 interference was a weak, but sharp, continuous ring. Similarly, in the X-ray diffraction photographs of two visibly decomposed pieces of ancient *Picea excelsa* and *Heritiera fomes* wood from Madhyamgram near Calcutta, the reviewer (Sen, unpub. a) has noted the slightly blurred appearance of the (002) reflections of the lattice of native cellulose. Marked loss of orientation and also broadening of the lines are clear. In similarly degraded wood of ancient *Heritiera fomes* from Jadavpur near Calcutta there is almost no trace of cellulose in the X-ray diffraction photograph, but a rather distinct ring of some inorganic matter which is not oriented.

It has been suggested by Wardrop and Preston (1949) that in any case the complete absence of diffraction arcs does not necessarily imply complete separation of the molecular chains of cellulose, for, according to Fankuchen and Mark (1946), diffraction arcs are not to be expected from micelles of less than a certain diameter (ca. 20 Å). So the presence of diffuse diffraction arcs in the X-ray diffraction photographs of some ancient wood (Sen, unpub. a) may not always indicate that the cellulose molecular chains are separated. But since the photographs in question are those of visibly decomposed wood, possibly the degradation process has decreased the size of the crystalline domains and at the same time destroyed their orientation.

X-ray diffraction photographs of well preserved ancient and buried wood samples, e.g., *Shorea robusta, Pinus sylvestris* (Sen

and Basak, 1955), *Bischofia javanica* and sound core of *Picea excelsa* (Sen, unpub. a), show very clear preferred orientation of various degrees of the cellulose micelles. The spreading of the equatorial arcs, which indicate micellar spiral angles with reference to the long axes of the cells, have been measured with accuracy. All these X-ray observations are consistent with chemical and physical data, and are related to the degree of preservation of the anatomical organisation.

It has been observed that the degree of preservation of various ancient and buried wood samples depends more upon the conditions in which samples were left and their intrinsic nature (e.g., density, degree of protection offered by lignin to cellulose, etc.) than the mere " time " factor.

SUMMARY

In recent tracheids heavily infected by brown rot fungi, the fine cellulosic wall structure is gradually destroyed. In some cases, however, slight infection by a brown rot fungus may improve the crystallinity and degree of orientation of cellulose in wood. The true skeletal cellulose structure of the cell-wall may be preserved when a fungus, chiefly destroying lignin, works on the cell-wall. The cellulose orientation may generally determine the plane of enzyme action of the fungi. Such is the nature of microscopic and submicroscopic recent wood structure variably infected by fungi.

In ancient fibres and tracheids, where cellulose is destroyed, the original microscopic structure is left in the form a textural pattern. Mysterious lignin [3] faithfully preserves the characteristic microscopic pattern after the breakdown of the cellulose and thus reveals the texture of some of the entrancing beauty of ancient plant life, down to the very smallest limits of visibility. The same lignin often persists in petrifactions, when the original structural units are considerably modified, and thus perhaps greatly helps in the preservation of the general anatomical plan of the original plants.

Palaeobotanists, therefore, are greatly indebted to lignin. The cellulose usually controls the texture in the lignin, which carries its patterns through the ages. But when cellulose itself is pre-

[3] The nature of equally mysterious cutin forms the subject matter of a separate paper (Sen, 1954, unpub. c).

served on occasions, its wonderful architectural plan is more easily and truly revealed with all its wonderful accomplishments to the students of submicroscopic morphology. Such cases have also been recorded.

STAGES OF FOSSILIZATION AS SHOWN BY ANCIENT AND BURIED WOOD

The study of ancient and buried wood leads to speculation regarding coalification and petrifaction of woody tissues.

Varossieau (1949, 1950) has found striking similarities in degraded gross structure of some buried coniferous wood from Rotterdam pilings with a few subfossil and fossil woods. This author is convinced that the characteristic changes in the cell-walls of his wood specimens appear as " identical with the first of a series of changes, which wood undergoes during conversion to coal or during petrifaction ". Similarly, the ancient wood from the Fishweir and various plant remains associated with it have been considered to be of " unusual interest in delineating very early stages in the long and complex series of events which ultimately result in the coalification of plant tissues ". The series of changes which affect the cell-wall structure and composition in degraded wood, as already indicated in this review, " appear to represent fundamental phenomena in fossilization " (Barghoorn, 1949b).

X-ray diffraction photographs of the ancient wood of *Heritiera fomes* (collected from Jadavpur) and *Shorea robusta* show a distinct non-oriented inorganic ring in the former, and scattered spots in the latter. Chemical analyses of almost all ancient wood species so far investigated by the present reviewer (Sen, unpub. a) and others (for details see Jahn and Harlow, 1942; Freeman, 1946; Varossieau and Breger, 1952) also show that there is an increase in ash content. This is partly the result of the accumulation of mineral and other extraneous substances due to infiltration or like processes. Such accumulation of mineral matter, etc., is natural during petrifaction of plant tissues for " fixation " of structure. But very little is known about the processes of such mineral infiltration and subsequent precipitation (Arnold, 1941, 1947; Barghoorn, 1952). The possibility that a catalyst, as NaCl, may help in silicate formation is also worthy of consideration (Blyumen, 1945).

The disoriented inorganic ring and the irregular inorganic spots, as referred to in the foregoing paragraph, at an early stage of fossilization may result from a small amount of mineral precipitates within the empty cell cavities. At such "incipient stage of fossilization" the minerals may not be properly oriented. But in advanced stages, when the cell cavities are being gradually filled up, the minerals infiltrating the tissues may be preferentially oriented in certain planes as a result of consolidation and newly acquired structural internal equilibrium of the developing pertrifaction. This has been substantiated, as indicated in Part II, by the presence of preferred fabric patterns of quartz in some Indian silicified wood (Sen, 1955, unpub. b).

II. Fine Structure in Fossilized Plant Derivatives

FINE STRUCTURE IN PETRIFACTIONS AND COAL

Petrifactions and coal are two suitable materials for studying the fine structure in fossilized plant tissues. Before and during fossilization, plant materials undergo a series of unknown phases of metamorphosis. It is interesting, therefore, to compare the fine structure in fossilized cell-walls with that of the probable original structure. In such materials the original cell-wall constituents may be partially present in unmodified form or they may be so altered that an entirely new structural organization may be present. In petrifactions, sometimes the whole original organization is replaced by inorganic units. The general anatomical organisation is variously modified or preserved in these fossils. Under high resolution even the gross organisation of wall structure is revealed in some instances. Total disorganization and loss of original plant structures are not uncommonly found.

PETRIFIED WOOD. Recently Wesley and Kuyper (1951) made electron microscopic observations on the structure of scalariform tracheids of *Lepidodendron vasculare*. These authors concluded that the transverse bars and the vertical connecting threads are part of one and the same wall structure. They have also concluded that the vertical threads form part of the secondary wall deposits as previously claimed by several authors who used ordinary microscopic methods. This is an instance where submicroscopic morphology may confirm microscopic observations. It has also been

noted by Wesley and Kuyper (1951) that the two systems of vertical threads belonging to adjacent tracheids, which cross the pit areas between the transverse bars, " bear no relation to one another, either in their pattern of branching or at their points of attachment to the transverse bars ". This makes the smaller pit areas virtually blind in some parts.

In Indian Tertiary petrified wood of *Glutoxylon,* containing lignin residues but without cellulose, the present reviewer (Sen, 1955) has observed excellent super-micelles in perfect alignment. These super-micelles, even though consisting mainly of lignin, most likely indicate the original line-up of the cellulose. Thus perhaps the original fine microscopic texture of the mother substance may be retained in petrified wood where sufficient lignin is retained.

The petro-fabric patterns in a number of Indian Palaeozoic and Tertiary silicified wood samples have recently been studied by using the Universal stage. In such growth fabrics the preferred orientation of the structural units (quartz in these cases) is possibly mainly controlled by a pre-existing pattern, possibly of the original cellulosic structural framework of the cell membrane. Following an indigenous method, Turnau-Morawska and Jahn (1952) prepared orientation diagrams of quartz in the silicified trunk of *Araucarioxylon schroellianus* appearing in situ in the Permian sediments near Chrzanów. It has been concluded that during crystallization of silica in wood cells, some phenomena of deformation in the crystalline lattice may occur, which appears in the strain shadows of quartz grains. The resistance of the membrane produces stresses and strains, the result of which is a preferred optic orientation of quartz grains, similar to that appearing in crystalline schists. Another interesting feature is that the quartz grains are usually elongated in the direction of the cell-axis. The phenomenon of secondary growth of quartz grains has been interpreted as an analogous process in the silicification of wood and in the diagenesis of sandstones, where silica is infiltrated in place of an older cement of different chemical nature or filled the pores between the grains. Whereas the centre of quartz grains in sandstones is evidently older than the rim, the age relation of quartz in the cell and membrane are not known.

The orientation of quartz thus reveals the nature of the fine inorganic architectural pattern in mineralized wood samples. Besides

quartz, which is the principal component of Indian and Polish silicified petrifactions studied, eight other minerals, heretofore unrecorded in petrifactions, have been identified by means of X-ray diffraction method (Mitra and Sen, unpub.). Among these minerals, amesite, serpentine and α-carnegieite are dominant. Such is the nature of inorganic structural organisation in petrified wood.

COAL AND OTHER CARBONACEOUS MATERIALS. Almost all palaeobotanists and coal chemists believe that cellulose and lignin are usually the principal mother substances of coal. The process of coal genesis is possibly influenced to some extent by interactions of these with vegetable proteins. The long chain macromolecules of cellulose and protein, under certain conditions, may fold up and associate together to form colloidal micelles or structural units of coal, as proposed by Dr. Bangham. The micelles are bound to their neighbours by physical rather than chemical forces. Bright coal or vitrain at least is in such colloidal condition. An alternative method of the building process has also been suggested. Possibly the " durain contains vitrain or bright coal in intimate admixture (on or above the colloidal scale) with mineral matter and opaque constituents of unknown properties ". This subject has still to be investigated (British Coal Util. Res. Assoc., Quart. Gaz., 1950).

Humification and coalification of plant tissues involve complex changes, most of which are not known and are in speculative stages. So, elucidation of the present knowledge about the structure of coal and other fossil fuels may be an interesting approach to the problem.

The colloidal micelles in coal referred to vary considerably in size, depending upon the rank of coal. They are measurable in terms of Ångstrom only. But Thiessen (1932) recognised microscopically visible micelles (designated as super-micelles), measurable in microns in thin sections of coal under the Spierer lens.

It would be difficult to demonstrate plant micelles in coal by means of X-rays, owing to the presence in coal of much inorganic materials. These inorganic constituents produce their characteristic patterns in the X-ray diffraction photographs and thus mask the presence of plant micelles in coal, even when they are present. Probably the low angle scatter work of D. P. Riley (1944a), which is now being continued by others, may throw further light on the colloidal structure of coal, but there are difficulties in obtaining definite results, owing to the heterogeneous character of

most coals. Even though it may be difficult to recognise original plant micelles in coal because of its heterogeneous nature, it would be worthwhile to find evidence that the "molecules in coal are oriented with some relationship to the original structure of the plant tissues. By such studies one might perhaps obtain new information with regard to the nature of chemical processes which underlie the transformation of original plant constituents in coal" (Goldschmidt, 1944).

Structure of Coal. Thiessen's (1932) microscopic structural units in coal are known as "super-micelles", or "giant molecules" as the designation implies, which are at the lower limit of visibility under an ordinary microscope. The colloidal structural units or the micelles come next in order of size. This has been pointed out by Dr. Bangham and his associates. After this the still smaller units, characterised by a greater degree of order and regularity, are Professor Riley's crystallites, though it is known that the structure so far interpreted from X-ray diffraction photographs may possibly be only a relatively small portion of the whole coal substance (Proc. Ultra-fine Structure of Cokes and Coals, B.C.-U.R.A., 1944). Some of the interesting observations on the submicroscopic morphology of coal have been cited by Horton, Randall and Aubrey (1944). But these authors have not attempted to synthesize such observations into a structure for coal. Moreover, the literature which appeared since the publication of Horton, Randal and Aubrey's article now deserves special consideration in the light of previous work. It should be noted in this connection that the studies on coal structure are mostly based on vitrain, since it is the only more or less homogeneous coal constituent.

The chemical structure of vitrain coal is of the nature of a high polymer or condensation product formed from residues of a simpler nature. The difficulty in assigning a structural formula to coal results from the increasing oxygen content with maturity.

Since his death, Dr. Bangham's whole concept of coal structure (more or less at a colloidal scale) (Bangham, 1944, 1950; Bangham, Franklin, Hirst and Maggs, 1949) has been summarised (see B.C.U.R.A. Quart. Gaz., 1950). His simple model of coal structure consists of a closely packed group of somewhat spherical units or micelles, averaging less than one millionth of an inch in diameter. Possibly coalification involves compaction of an assembly of

micelles which result in the growth of the contact regions with a consequent decrease in pore dimensions. This is due to the influence of pressure and temperature in geological times. The structural units are thus stabilised by chemical condition and cross linkage internally. It remains ambiguous, however, whether the " chain like compounds persist in any form within the mature coal substance itself, or whether they have formed by the condensation process large three-dimensional macromolecules ". Most of the materials must have condensed into polycylic molecules, as is evident from the C/H ratio and the progressive aromatisation of coal oxidation products with maturity.

To the X-ray workers the picture generally appears as a honeycomb-like layer-plane structure consisting of flat aromatic lamellae which are built up almost exclusively of carbon atoms forming " turbostratic " crystallites with layer spacing of 3.5 Å in association with the relatively irregular bitumen lamellae (with a layer-plane spacing of up to 4.5 Å) (Blayden, Gibson and Riley, 1944). But how carbon and bitumen are blended together is not understood. Coal thus forms a " turbostratic " system (indicating also random orientation of structural units) as present in carbon black with two-dimensional repetitions. This simulates graphitic structure without the third dimension characteristic of graphite, and consequently the stacked formation of graphite-like layers arranged equidistant and parallel but with a random orientation about the layer normal result. The coal fractions are also crystallographically similar, forming " turbostratic " systems. Coal and simple pure carbon compounds mainly differ in the presence of bitumen in coal which involves mobility. Such a picture has been admirably summarised by Kipling (1949) and others.

The amount of bitumen in coal is very small (Moore, 1940). It is bitumen which is responsible for the colloidal behaviour of coal. The bitumen is an organo sol consisting of two phases—an oily phase and a dispersed phase (micelle phase). These micelles are giant molecules, the high polymers of the isogel type in which the innermost portion is a monomer, the next layer is a dimer, the next a trimer, and so on—an onion-like structure. Thiessen (1932) has claimed to have photographed these micelles under the Spierer lens. The important gap in our knowledge is about the arrangement of atoms inside bitumen.

Recently Mitra (1953) has examined the X-ray diffraction bands of different intensities of Indian vitrain, and compared his results with those of previous workers. His "experimental observations cannot be satisfactorily explained on the basis of the accepted structure of graphite". It has been shown by Nelson (1954) from low-angle scattering of vitrinite that the "evidence for the existence of submicroscopic crystallites of graphite-like nature, even in the case of the highest rank bituminous coal" is lacking.

The different points of view of the X-ray workers on coal has recently been discussed by Mukherjee (1954). In another excellent article Brown and Hirsch (1955) have summarised recent infra-red and X-ray studies of coal. The two methods are complementary because the nature of the disordered material in coal can be ascertained only from infra-red studies when the X-ray technique is not helpful. X-ray studies, on the other hand, reveal the changes in the chemical structure with carbon content and the packing of the condensed aromatic layers. These layers have no definite orientation relative to each other about an axis normal to their plane (turbostratic packing), and they may be displaced parallel to each other. The layers may occur singly or in groups of two or more. With increasing carbonisation the order improves, as a result of which two-layered parallel structure is most common in all coals. The layers are more or less oriented parallel to the bedding plane. The details of the packing of the layers and their relation to carbon content, existence of pores, etc., have also been considered by the same authors.

The differences in density of all these carbonaceous substances add to the difficulties. Blayden, Gibson and Riley (1944) have established the presence of a layer-plane structure in a wide range of materials from "peat to bituminous coals and from bituminous coals to anthracite on the one hand and coke on the other".

More recently Riley has expressed doubts in the concept of a micellar structure for high rank Carboniferous coal. It has further been suggested that residual coal (Restkohle) possibly has a highly disordered three-dimensionally cross-linked structure. Nelson (1954) feels that, although the low angle scattering effects of X-rays do not provide direct evidence as to the existence of a micellar structure, there is apparent inconsistency with such a view.

Coalification involves carbonisation of vegetable matter consisting of cellulose, lignin, vegetable protein, etc., into rigid " turbostratic " systems. One or more of these mother substances form large bitumen molecules which are relatively susceptible to thermal influence; the rest are developed into carbon-like crystallites forming a rigid system. This association of bitumen and carbon crystallites constitutes the characteristic structural feature of bituminous coal.

Microscopic Studies on the Super-micellar Architecture of Coal. Microscopic study of wood structure of recent materials is usually made in terms of its submicroscopic morphology. Application of the same principle in evaluating a structure for coal is full of pitfalls, especially when the composition and nature of structural units involved are different.

In the excellent Spierer pictures taken by Thiessen (1932) and subsequently reproduced by Seifriz (1936a, b), Kreulen (1948) and others, the ligno-cellulose super-micellar patterns similar to those in modern plants are present in woody cells in peat, lignite and bituminous coals, and the super-micellar arrangements, as preserved in these fossilized materials, are similar to those of plant tissues from which the fossil fuels have been derived. According to Thiessen (1932), the original super-micellar arrangement, although more or less progressively disarranged in high rank coals, has been clearly and unmistakably shown. Therefore, preferred orientation of these microscopic structural units is rather common in coal. But the submicroscopic micelles in coal generally exist in " brush heap " order, as can be found from the available X-ray data [4]. This anomaly, as has already been pointed out, is due to the great differences in the structural units involved in the two methods of study. Moreover, it would be very difficult to obtain evidence of plant micelles in coal by means of X-rays, owing to the presence in coal of an appreciable amount of inorganic matter.

The late Dr. Charles Spierer of Geneva kindly sent me some excellent photographs of microscopically visible super-micellar images in samples of anthracite, coke and charcoal. He employed the very simple and safe method of reflected light for observation and subsequent photographing. The general trend of arrangement of the microscopic super-micelles in anthracite and coke thus reproduced

[4] However, it is not yet definitely known whether crystallite size and/or disorder are mainly responsible for the broadening of the diffraction lines.

appears to be highly consistent with the usual X-ray patterns of these materials. In the photographs of anthracite and coke the microscopic super-micelles are in " brush heap " order which simulates the usual organisation of the submicroscopic micelles in such materials, except in some anthraxylon investigated by Turner and Anderson (1931). The microscopic micelles in the photographs of charcoal are quite well oriented into parallel striae. The X-ray patterns of similar materials, e.g., fusain, exhibit localised spots of intensity, indicating fibre structure.

Charcoal, which shows regular arrangement of the microscopic super-micelles, may not produce preferred X-ray diffraction patterns under certain experimental conditions. The reasons for such an apparently anomalous situation have already been explained in the foregoing account. However, activated charcoal may show preferred orientation of the submicroscopic micelles.

Seifriz (1936a) believes that pictures of colloidal matter produced by the Spierer lens represent a true structure. He has actually shown that the diffraction lines can be wholly avoided under favourable conditions, viz., when the differences between the optical constants of the object and of the medium are small. He has also reproduced Spierer pictures of similar nature of coal and cellulose—" this is to be expected since the one material, coal, comes from the other, cellulose ". The question has been emphasized as to why any optical system, like the Spierer lens, should sometimes show similar, at times identical, structure in otherwise diverse materials. The best evidence of the reality of Spierer pictures (those in which artefacts are eliminated or reduced to a minimum and then distinguished from that which is real) is their reproduction by other methods. Scarth's photograph of protoplasm taken in light field and an X-ray pattern of the cell wall of *Valonia* by Astbury et al. are a few of the many cases where Spierer pictures are identical with the images formed by the aforesaid materials (Seifriz, 1936b). Similarly it has been shown by the present author (Sen, 1955) that the cellulose fibre of *Calotropis gigantea* and normal wood of *Heritiera fomes,* under the Spierer lens, produce super-micelles which are very regularly arranged parallel to the long axes of the cells, like the submicroscopic patterns of the same materials. The Spierer pictures of swelled cellulose fibre, shredded woody tissues in peat, ancient and degraded, and petrified woods, lignite and coal are likewise regularly arranged

with varying degrees of orientation. Most of these decomposed, degraded and partially or wholly petrified or coalified materials have retained the general high degree of orientation of the supermicelles wherever they are preserved. In a personal communication Professor Hickling also expressed his strong belief in the general soundness of Thiessen's (1932) conclusions.

So, even in a changing chemical set up, the original microscopic textural pattern in wood may be preserved in coal, at least to some extent. Similarly in fusinized tissues " the fidelity of morphologic preservation is sometimes very striking in comparison with the great alteration in chemical composition " (Schopf, 1948).

X-ray Diffraction Patterns of Coal and Other Carbonaceous Materials Which Do Not Show Preferred Structure. From 1929 to 1946 Mahadevan systematically investigated the submicroscopic morphology of various types of Indian coal and carbonaceous materials of Tertiary to Carboniferous age by employing the X-ray diffraction method.

Mahadevan's (1930) examination of graphitic anthracite and elaterite clearly brings out the absence of fibre pattern in these materials. Graphitic anthracite exhibits well defined haloes of carbon and ash, and elaterite gives a number of rings having close affinity to the higher members of the paraffin series.

The investigations on banded bituminous coals by Mahadevan (1935, 1940) reveal the presence of two diffuse haloes of varying intensities in vitrain, with particle size suggesting a colloidal nature for the band. Durain gives eight haloes, two of them the same as in vitrain. The superposition of the ash and graphite powder haloes in the vitrain pattern reproduces the pattern exhibited by durain. The X-ray patterns of dehydrated and devolatilised coals become sharper with less background scattering. Even so, none of such specimens exhibit any fibre pattern. This fact has since been substantiated by many authorities (for detail see Horton, Randall, and Aubrey, 1944; Proc. Ultra-fine Structure of Cokes & Coals, B.C.U.R.A., 1944, and others).

X-ray Diffraction Patterns of Coal and Other Carbonaceous Materials Which Show Preferred Structure. Turner and Anderson (1931) have noted that Pennsylvania anthraxylon produces " a true fiber pattern characteristic of a very finely divided material—that is to say, one in which the intensity maxima are broad and

diffuse connected arcs of diffraction haloes, in addition to the large scattering at small angles". The diffraction photograph is very similar to that of cellulose, and these authors assume that during coalification the anthraxylon particles maintained their original orientation. These authors, however, also got diffraction patterns in which the anthraxylon was not fibred. It presents the so-called "brush-heap" arrangement of the particles. The X-ray evidence of anisotropy in anthracite has been discussed also by D. P. Riley (1944a) and Knaggs (Cannon and George, 1944). The crystallinity study of certain high ash-content coals and baste by the X-ray diffraction method has also revealed "the orientation of the layer-minerals and the ordering of the coal substance" (D. P. Riley, 1944b). The reasons for the presence of ordered structural units in some coals (e.g., specimens of anthraxylon) and their usual absence from most coals (e.g., usually of bituminous type) have been discussed elsewhere in this paper.

In India Mahadevan (1930) made X-ray analyses of fibrous and non-fibrous peat. The latter variety presents only two haloes, both diffuse and ill-defined, but the former gives more weak (at the same time well-defined) haloes and localised spots of intensity characteristic of fibrous structure. It is important that a comparison of the spacings for durain and fibrous peat, and vitrain and non-fibrous peat, reveals that all the rings present in true coals (except those due to ash) are also present in the corresponding peats (with some increase in the spacing of each ring). Mahadevan holds that the differences in X-ray patterns between the two are mainly of structure, not of composition. These structural differences may also possibly involve micellar disorientation in addition to intra-micellar changes.

Mahadevan (1930, 1940) also examined fusain specimens giving eight sharp haloes; the inner ones produced localised spots of intensity characteristic of fibre patterns. It may be mentioned in this connection that Mahadevan (1946) found almost identical X-ray patterns in flax lignin and in three Tertiary coals. On a comparison of X-ray patterns for untreated flax and its lignin, it is seen that, except for the fibrous nature of untreated flax, the haloes in the two cases show great similarity. This shows that the submicroscopic structural patterns of fossil and recent lignin are remarkably similar although not quite identical. Retention of the original pattern in fossil lignin indirectly favours Thiessen's

(1932) observations. Thiessen holds that lignin retains the same general line-up as cellulose, even if the latter is destroyed, and as such the original micellar pattern of cellulose is maintained by lignin. This pattern is sometimes persistent. But when cellulose is present (Freund, 1952), possibly the original structure is retained to some extent. " Even unchanged cellulose has been reported in some coals " (Riley, 1944) where the plant micelles must have been largely preserved.

The X-ray diffraction data at hand, therefore, indicate the presence of preferred structure in some anthracite, baste, fibrous peat, fusain, etc., whereas such pattern is usually absent from bituminous and similar types of coal.

It may be suggested that high temperature and pressure are necessary for the origin of anthracite with high carbon and less volatile matter. This perhaps provides the necessary energy for retaining or forming the preferred orientation of the constituent particles in anthraxylon. With less temperature and pressure the potential energy for retaining the preferred structure may not be obtained, and as such, random orientation results, as in most bituminous coals. It has also been indicated by some contributors to a Conference on the Ultra-fine Structure of Cokes and Coals, B.C.-U.R.A. (1944), that the degree of order of the structural units usually increases with increasing carbonisation (i.e., rank) as present in coke. This effect is no doubt the result of metamorphic changes taking place subsequent to the initial aggregation of the ulmic-humic micelles in the aqueous media of the forest swamps. D. P. Riley (1944b) has suggested that the ordered structure in his specimens of high ash-content coal and baste is most probably due to the " ordering induced by the large flat surfaces of the layer-minerals, which would tend, once initiated, to extend a considerable way into the coal substance ".

Other Optical Studies on Isotropic and Anisotropic Coals. A more logical conclusion regarding the presence or absence of preferred orientation in coal, based on experimental evidence, has been reached by the contributors of a paper entitled " Optical Properties of Coals " presented to the Conference on the ultra-fine structure in cokes and coals (Cannon and George, 1944). These authors distinguished anisotropic from isotropic coals. They considered geological pressure to be the factor which might have been responsible for uniaxial negative birefringence. The birefringence

naturally refers to the presence of " platelets " of colloidal dimensions, small in comparison with the wave length of light, oriented parallel to the bedding plane. In optically isotropic coal the diffracting units are in " brush-heap " order, whereas in optically anisotropic coal the carbon layer planes are arranged parallel to the bedding plane and thus exhibit a preferred orientation. Such are the orientations of micelles in optically isotropic and anisotropic coals.

This idea was long ago supported by Ambronn and Frey (1926) on optical data, and has more recently been confirmed by direct X-ray diffraction evidences. A monochromatic beam of X-rays passing through chips of Indian bituminous coal (isotropic) produces diffuse diffraction rings of equal intensity and as such suggests a random orientation of the diffracting units (Mahadevan, 1935). As already stated, similar treatment of Pennsylvanian anthracite (anisotropic) has resulted in some cases in the formation of intensity maxima on the diffraction haloes, indicating a preferred orientation of the diffracting units (Turner and Anderson, 1931). Such experiments were repeated by Dr. I. E. Knaggs (Cannon and George, 1944) with the same results.

From the available data on the micellar structure of coals and optical results, Cannon and George (1944) suggested that a random orientation of parallel carbon layer planes results in an " open " micellar structure with a large internal surface in free burning coals. Loss of volatile matter leads to close packing of the micelles, resulting in increase in absorption coefficient and refractive index with simultaneous decrease in internal surface. In coking coals which are highly rich in carbon with minimum internal surface, the platelets are arranged parallel to the bedding plane with the development of optical anisotropy as a result of pressure. The orientation becomes more preferred in anthracite and the internal surface again increases.

Such appearance of preferred orientation, however, does not necessarily indicate that it is persistent after it is once laid down in the mother substance (i.e., wood, etc.), as postulated by Thiessen (1932). In the foregoing paragraphs the metamorphic changes involved in the formation of high rank coal have been discussed and show a re-orientation of the submicroscopic structural units with ageing and progressive carbonisation.

Coal and Its Carbonised Mother Substances. It is necessary to point out first that it is extremely difficult to compare certain carbonised materials, e.g., those of lignin, cellulose protein, etc., with coal because one has to reckon with a very wide variety in what we normally call " coal " and with the very complex chemical composition of coals. Such carbonised substances as lignin, cellulose and similar carbohydrates may be compared to coal after it is carbonised.

Dr. Kipling indicated the two essential stages in any carbonisation process: (*a*) formation of an aromatic structure from the original material, involving loss of oxygen and hydrogen; this occurs mainly below 700° C., the lowest at which coal is formed naturally; (*b*) re-arrangement of the aromatic structures above 700° C. The behaviour of raw materials can be very different during the first stage, but similarities are found in the second stage. They do not, however, come into consideration, for coal does not undergo such high temperature in its natural course of formation. A wide variety of carbohydrate materials—various kinds of wood, lignin, cellulose and sucrose—have been used for making adsorptive charcoals, in which such differences seem to result from slight chemical changes and to be a reflection of physical structure. Krevelen (1952) has recently discussed the changes in the elementary composition and structure of plant substances during coalification.

Relationships of Coal to Cellulosic Structure: Persistence of Cellulosic Micelle in Coal as a Possibility. Berl and Koerber (1940) and associates regard cellulose as the principal mother substance of humic acid. Intensive investigations on the c-parameter of lignin crystallites suggest that the layer-plane spacing in lignin itself is already less than that in bitumen. It appears, therefore, more logical for cellulose than lignin to be the mother substauce of coal (from Riley, 1944). Riley (Horton, Randall and Aubrey, 1944) is also inclined to believe that the bitumen in coal originated from cellulose, not from lignin. Earlier Berl and Keller (1933) discredited the lignin theory of coal formation on the ground that cellulose can be converted into coal in the laboratory.

Although some of these carbonised cellulosic products exhibit swelling and caking properties, on X-ray analyses they are more closely related to peat and brown coal than to bituminous coal

(Riley, 1947). Moreover, cellulose is aliphatic and composed of furan-like units, and as such can not give rise to any aromatic structure at different stages of coalification, as is characteristic of bituminous coal.

Spierer believes that the cellulose molecule is destroyed by carbonization and that, as a result, only carbon is left. But the arrangement of the carbon molecules inside each colloidal particle of cellulose remains almost unchanged and thus the micellar arrangement of cellulose persists after carbonization.

Relationships of Coal to Lignin Structure: Persistence of Lignin in Coal as a Possibility. Agde and Jodl (See Hägglund, 1951) have shown that X-ray diagrams of the non-bituminous constituents of lignite exhibit fundamental similarities to those of lignin (Jodl, 1941). This has been regarded by them as a proof of the lignin theory of coal formation (Agde, Schurenberg and Jodl, 1942) of Fischer et al. (Hägglund, 1951).

Sedletzky and Brunowsky (1935) found a close, structurally genetic relationship between lignin and humic acid, lignite, anthracite and graphite. They believe that with increasing rank the lattice spacings approach more closely those of graphite. Sedletzky (1939) showed that the basis of structure of all coals is the aromatic ring. He is unable to find evidence of a fibre or cellulosic structure in any coal, and can not detect the carbohydrate or protein structures in the coals he examined. The pattern for lignin has been found to be similar to that for coal.

More recently Breger (1952) has suggested that cellulose is probably removed biochemically from sediments soon after plant fragments are deposited. But the lignin possesses a structure (e.g., that of Russell lignin) which may be converted into humic substances, formed during coalification, by chemical agents. This author has further made some proposals to show the changes which may occur in the lignin molecule to convert it into a series of related compounds collectively known as "humus". Infra-red absorption spectra also show the structural similarities between lignin and humic acid.

Lignin itself is aromatic, and its char as well as that of glycine yield almost similar results. It has also been shown by Blayden, Gibson and Riley (1944) that under variable experimental condi-

tions, cellulose, lignin and glycine exhibit constant c-dimensions of crystallites which, however, vary in coals (except in fusains). But the lignin char often differs in crystallites with a dimension larger than those of the corresponding cellulose char crystallites, at low temperature. Initially lignin crystallites in many respects simulate those of graphite, and differ from cellulose (without condensed aromatic ring system) in having a highly aromatic ring system. With progressive carbonisation of lignin the structure of hexagon planes appears to become simpler, and ultimately only the simplest radicals remain along the edges of hexagon layer planes (Riley, 1939).

From thermographic study Breger and Whitehead (1952) have concluded that the fundamental structure of lignin is present in low grade coals but subsequently disappears with the condensation or polymerization of the coal structure. The cellulosic structure is rather obscure in peat and lignite, and appears to be totally absent from high grade coals.

Obviously the original cellulosic structure is completely lost in coal. The micellar pattern formed by cellulose in wood and presumably retained in coal, according to Spierer pictures, has probably been preserved by some lignin residue or its product which has retained the same general line-up as the cellulose, even after the latter is destroyed. Such an explanation, offered by Thiessen (1932), may be substantiated by the identical nature of fossil and recent lignin (Mahadevan, 1946), and by the similar trend of arrangement of super-micelles in Spierer pictures of recent, ancient and petrified woods, and in lignite and coal.

SUMMARY

"Brush-heap" X-ray diffraction patterns are now known in coals, except fibrous peat, fusain, some anthracite, etc., where the micelles assume preferred orientation. Microscopic Spierer supermicelles are, however, found well oriented in the secondary cell-wall of recent plants, peat, lignite, sub-bituminous and bituminous coal, charcoal, etc. But the structural units involved in the two methods of study (microscopic and submicroscopic) are essentially different. Therefore, this does not entail any controversy. It is well known that micelles are present in wood and throughout the stages of carbonisation in varying degrees of orientation and dis-

orientation; but, as has already been stated, the micelles demonstrated by X-ray diffraction and the Spierer lens are not of identical structures.

According to Thiessen, the Spierer pictures exhibit the patterns of the persisting fine lignin residues which once ensheathed the cellulosic chains in the original woody mother substances of coal. Obviously, such pictures may reveal only the super-micellar texture, i.e., the pattern of arrangement of the structural units, of the original cellulose. Similarly, in ancient fibres of *Heritiera fomes* and tracheids of *Picea excelsa* the original cellulose framework is destroyed, and possibly the fine ensheathing lignin residues produce the original super-micellar texture, not the micellar structure. So, whenever a regular arrangement has been found preserved in coal, etc., under the Spierer lens, this usually depicts a micellar texture probably inherited from the mother substance. Such pictures of lignin can not obviously be produced in the X-ray diffraction photographs. Hence such materials exhibit a " brush-heap " arrangement of sub-microscopic structural units.

It may also be suggested that if there is any preferential orientation of the graphite crystal lattice in coal, whether located in discrete micelles or otherwise, then, theoretically, it should be revealed in the ordinary Bragg diffraction pattern. If, however, the crystallites are very small, the indications may be obscured by the broadening of the diffraction lines. The small angle scattering of the monochromatic rays near the centre of an X-ray diagram may reveal new facts regarding the precise nature and orientation of the submicroscopic structural units in coal. The heterogeneous nature of coal is also perhaps responsible for the absence of a pattern true of plant micelles, even when they are present in coal.

It appears that the products of carbonisation leading to coalification usually more closely resemble aromatic lignin structure than that of any other wood constituent. The lignin structure has also been found convertible into humic substances. The structural similarity between this material and that of humic acid has been suggested recently.

Pictures of the physical structures of vitrain coal have been drawn by some leading authorities on the basis of submicroscopic structural units of different size. It has been felt that the " initial fundamental attack on coal structure has been reached ". The colloidal and crystalline structure for coal is now based on an enor-

mous amount of experimental data. There is a growing idea among some workers, including Dr. Riley, that a micellar structure for coal is probably incorrect.

It is perhaps premature to construct a complete picture out of the results of investigations carried out by botanists, chemists and physicists so far because the data are insufficient. But a start, as outlined in this review, is obviously necessary to find out which data are missing. The extensive work on the carbonisation of coal and its probable mother substances are, at least, the result of recent investigations.

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THE PLANT CENTROSOME AND THE CENTROSOME-BLEPHAROPLAST HOMOLOGY [1]

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Introduction

The centrosome problem in plants and the centrosome-blepharoplast relationship which was once much debated have been investigated only sporadically in recent years. The work of Ikeno (1903a, b, 1904, 1905, 1906) and Sharp (1912, 1914, 1920) contributed much to the current acceptance of the generalization that the centrosome is present in animal cells and in plants possessing motile male gametes. Most texts in current usage subscribe to this idea, stating it in broad or in more or less specific terms. This generalization includes all groups of plants from the Thallophytes through the vascular cryptogams to the Ginkgoales and Cycadales. Where more specific descriptions are used, it is pointed out that the "centrosome" of many of these plants is present only in the spermatogenous tissue, sometimes only in the last cell generation, where it functions in the development of the cilia or flagella and is therefore called a "blepharoplast" by botanists. This, it is held, does not alter the fact that blepharoplasts are centrosomes because, as Sharp (1920) stated, the blepharoplast is ontogenetically and phylogenetically a centrosome.

It is the purpose of this paper to consider the evidence for the presence of the centrosome in plant cells and to estimate the validity of the centrosome-blepharoplast homology. Most of the author's research has been concentrated upon a selected few of the Hepaticae, but treatment of these problems can not be concerned with this group alone, for much of the speculation which is significant for this problem was formulated upon evidence secured from observation in other plant groups. Nor would it be possible to eliminate from discussion all evidence involving animals, for it was in the animal cell that the centrosome was first discovered and in which the first problems arose which were later to impinge upon the speculation of the botanical cytologist.

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Review of Literature

Centrosomes were first seen and described by Flemming in 1875 in the egg of the fresh water mussel, *Anodonta,* and in 1876 Van Beneden discovered them independently in Dicyemids. In the following year Van Beneden and Boveri separately identified the centrosome in *Ascaris* as a permanent cell organ, and in 1888 Boveri coined the name "centrosome" (E. B. Wilson, 1928). Subsequent investigation established its wide-spread occurrence in a variety of animal organisms and many diversified cell types. Although there have arisen, from time to time, disputes as to the reality of this structure or to its function (Schrader, 1953; Fry, 1929), and despite the fact that it has been reported as having a considerable diversity of morphological form, the presence of a true centrosome in animal cells has never been seriously questioned. White (1950), however, has pointed out that the centrosome is by no means universally present in animal cells. Shortly after the discovery of their presence in animals, centrosomes were reported to be in plant cells as well, and soon the results of numerous investigations seemed to indicate that centrosomes exist as definite components of plant cells (Guignard, 1891; Wager, 1893; Farmer, 1893, 1895; Hirase, 1894; Schaffner, 1894, 1897a, b, 1898, 1901; Humphrey, 1895; Webber, 1897a; Fulmer, 1898, 1899; Trow, 1904).

Further investigations, however, indicated that in seed plants at least, no centrosome is present (Strasburger et al., 1897; Nemec, 1901; Gager, 1902; Mottier, 1903; Lawson, 1903; Koernicke, 1903; Merriman, 1904). Some authors, nevertheless, held that the absence of centrosomes from higher plants was not definitely established (Guignard, 1898; Chamberlain, 1898).

In 1894 Hirase found centrosome-like bodies which arose de novo in the generative cell of *Ginkgo* and termed them "attractive spheres". Webber (1897a) first discovered very large centrosome-like bodies in the pollen tube of *Zamia* and described their growth, structure, segmentation and independence of the spindle formation. In a later paper Webber (1897b) described the development of an extended helical band which developed from the outer boundary of the centrosome-like body, and from which the cilia developed. Almost simultaneously with the appearance of Webber's second contribution, Belajeff (1897a, b) published two

preliminary papers describing the presence of a cilium-forming organ, which he called a " Nebenkern ", in the androcyte of the Filicineae and the Equisitineae. In a third paper Belajeff (1897c) homologized the cilium-producing body (blepharoplast) with the animal centrosome, and finally, in 1898 he reported the blepharoplast first appearing in the androcyte of *Equisetum*. In *Gymnogramme*, however, he said it first appeared in the androcyte mother cell. These cell organs Webber considered to be identical with the similar structures in *Zamia* and *Ginkgo*. Since no trace of them could be found in the androcyte mother cells, either in the resting stage or during mitosis, it appeared that they arose in the androcyte.

In a third preliminary paper Webber (1897c) reported finding in *Ginkgo* the centrosome-like bodies described by Hirase (1894), and pointed out that undoubtedly they were the same as the centrosome-like bodies in *Zamia*. Since Webber considered these bodies distinct from typical centrosomes in their main function, namely, that of forming the cilia of the spermatozoid, he designated them " blepharoplasts ". Hirase (1894), in concluding that the attractive spheres of *Ginkgo* should be considered centrosomes, also noted that they were different from any type of centrosome theretofore described. This distinction was made on the basis that they were always found a certain distance from the poles of the spindle and that they did not divide into two daughter spheres during mitosis.

In a short paper early in 1898, Ikeno announced the occurrence in the androcyte of *Cycas revoluta* of cilia-forming organs like the blepharoplasts of *Zamia* and claimed that these and the Nebenkern of the Filicineae and Equisetineae were nothing but centrosomes.

Chamberlain (1898) stated that it seemed probable that a thorough investigation of karyokinesis and of the formation of cilia in the lower plants might support the theory that the blepharoplast is a centrosome.

Although admitting that all earlier observations upon the presence of attractive spheres and centrosomes in various vascular plants might be regarded as inexact, Guignard (1898) was convinced that the bodies described and figured by Webber in his report on *Zamia* were centrosomes.

In the same year Shaw (1898) described the occurrence of

blepharoplasts in the androcyte mother cells of *Onoclea* and *Marsilia* which developed into the cilia-bearing band, as in the cases described by Belajeff. These arose de novo, divided, increased in size and moved to a position near the place where the poles of the future spindle would be located. Shaw did not believe these bodies to be centrosomes or derivatives of them.

The following year Belajeff (1899) brought forth further evidence that the blepharoplast of *Marsilia* must be considered a centrosome, on the basis that in *Marsilia* the blepharoplast not only occupied the pole of the spindle but evidently took part in the spindle formation in all but the first of the four divisions which produced the androcytes.

E. B. Wilson, in the first edition of his text (1896), regarded the blepharoplast as the homologue of centrosomes or of centrospheres. He cited the later studies of Shaw and Belajeff on the blepharoplasts in *Onoclea* and *Marsilia,* which studies he felt left no doubt that these bodies are to be identified with centrosomes.

Webber's paper (1901) on spermatogenesis and fecundation in *Zamia* was published late in 1901 and contained a section devoted to the blepharoplast-centrosome problem. Among other observations, he noted that blepharoplasts differ from centrosomes in the following ways: (*a*) in not forming the center of an aster at the pole of the spindle but being located entirely outside the spindle in *Zamia, Ginkgo* and *Cycas;* (*b*) in having no connection with spindle formation; (*c*) in being limited to the division of a single cell, with no similar organs known to appear in any other stage of the development of the plants; and (*d*) in having a function differing from that of any typical centrosome, so far as known in plants.

Webber accounted for Belajeff's observations (1899) that blepharoplasts occur in *Marsilia* a few cell generations earlier in the spermatogenous tissue, on the basis that the 16 androcytes arose from the successive divisions of a central cell. This cell was thought to be homologous to the central cell of the male gametophyte of *Zamia.* It was, therefore, not illogical to expect blepharoplasts or their rudiments to be present in all of the intervening cells between the central cell and the androcytes, as these cells may be considered potential androcytes.

Davis (1901) noted that there had been a retreat from the views held generally previous to 1896 regarding the presence of

centrosomes in the cells of Pteridophyta and Spermatophyta, and that the belief of most plant cytologists was that neither centrosomes nor asters are present in any cells of these plants.

In 1900 Strasburger, as cited by Davis (1904a, b), reviewed swarm spore and spermatozoid formation and concluded that the blepharoplast of spermatogenous cells is distinct from a genuine centrosome or centrosphere. He traced its origin back to the "Mundstelle", from which originated the cilia of the swarm spores in such algae as *Cladophora* and *Oedogonium*.

In 1903 Ikeno, who previously had been of the opinion that the blepharoplast of the cycads and of pteridophytes is actually a centrosome, published his observations upon the spermatogenesis of *Marchantia polymorpha*, particularly on the centrosome and blepharoplast. In all divisions of the spermatogenous tissue he found centrosomes occupying the spindle poles and indicated their possible origin from the nucleus. He found a centrosome in the cytoplasm next to the nucleus and described its division and the subsequent movement of the daughter centrosomes to the poles, where they apparently took part in the formation of the spindle. They disappeared at telophase but reappeared at the prophase of each successive division. In the last division, however, in which the spindle was arranged along the diagonal of the mother cell, consequently forming two triangular androcytes not separated by a cell wall, the centrosome did not disappear but persisted and subsequently functioned as the blepharoplast. In the androcyte the blepharoplast migrated to one of the acute angles of the cell where it elongated somewhat, gave rise to the cilia and finally became applied to the nucleus through the medium of a body which Ikeno termed the "cytoplasmatischer Vorsatz". Ikeno also noted the appearance in the cytoplasm of a larger body, the "Nebenkorper", about the time the centrosome moved into the acute angle, and finally noted its disappearance when the blepharoplastic band was formed.

As a result of his subsequent researches in the mosses, *Atrichum angustatum* and *Poganatum rhopalophorum*, and the hepatics, *Makinoa* and *Pellia*, and noting Chamberlain's report in 1903 that there are no centrosomes in the earlier stages of spermatogenesis in *Pellia epiphylla*, Ikeno (1904) was convinced of the absence of centrosomes from these forms. He concluded, there-

fore, that, although centrosomes are constantly present in the antheridium of *Marchantia,* in other bryophytes they had gradually disappeared and now occur in only a few cell generations.

Mottier (1904) reported that the blepharoplast of the spermatozoid of *Chara* arises in the cytoplasm at the surface of the androcyte cell as a delicate thread-like differentiation which appears to be a modification of the plasma membrane. He further reported that no centrosome-like body is to be observed. Mottier was inclined to disagree with Ikeno's theory regarding the centrosome-blepharoplast relationship.

Much of the research on the centrosome after 1900 was centered in the Hepaticae, probably because they were considered to be derived from the Algae, where instances of true centrosomes were reported, and because they were thought to have been the progenitors of the vascular plants. Moreover, Ikeno's work with *Marchantia* had appeared to provide a final piece of evidence that the blepharoplast is of centrosomal origin, inasmuch as he repeated Belajeff's conjecture that the blepharoplasts of the bryophytes, pteridophytes and gymnosperms are ontogenetically and phylogenetically centrosomes. Schottlander in 1893 (Sharp, 1912) had been the first to report centrosomes in the spermatogenous cells of liverworts, and Van Hook (1900) and Mottier (1898) had reported their presence in vegetative tissue. In 1905 Miyake, a compatriot of Ikeno, disputed Ikeno's observations on *Marchantia.* He stated that he was unable to find centrosomes at the spindle poles of the earlier divisions in the antheridia of *Marchantia polymorpha,* but he noted a deeply stained body at each pole of the spindle in the last division, which he concluded was the blepharoplast. He stated that the situation in *Fegatella conica* is similar to that in *Marchantia.* He also reported the absence of centrosomes from any of the divisions in *Pellia, Aneura* and *Makinoa.* In *Makinoa* he noted that just prior to the last division, a group of granules is to be found some distance from each pole and conjectured upon its possible function later as a blepharoplast.

Ikeno (1905), in a reply to Miyake, accused him of poor technique and faulty observation, and reaffirmed his own statements regarding the presence of centrosomes at all spermatogenous divisions.

In 1907 Escoyez, contrary to Ikeno's observation, found no

centrosome-like body until the prophase of the diagonal division of *Marchantia polymorpha*. A darkly stained body appeared in each corner of the androcyte mother cell and was located at the pole of the spindle. A body of similar appearance was found to occur sometimes, in addition, in each corner of the cell not occupied by the spindle, according to Escoyez. He found no evidence of the nuclear origin of either of these bodies but stated that those occupying the poles of the spindle remained in the sperm cell and functioned as blepharoplasts. In *Fegatella* the last division was not observed, but no centrosomes were found in the earlier divisions. Escoyez's opinion was that the centrosome-like bodies present in the last division of *Marchantia polymorpha* were not true centrosomes functioning suddenly as blepharoplasts, but, instead, were organs sui generis of the antheridial cells. He argued that they play no role in karyokinesis and felt that the fact that they occupied the poles of the spindle was not an indication that they took part in spindle formation.

On the other hand, Schaffner (1908) corroborated Ikeno's observations in regard to centrosomes almost completely, only failing to state how the bodies originated and whether they persisted throughout all the phases of karyokinesis.

In other liverworts also there have been varied reports. Farmer and Reeves (1894) reported centrospheres occupying the spindle poles of the first two or three divisions of the germinating spores of *Pellia* and noted that these centrospheres became fainter with each division and finally did not appear at all. In the following year Farmer (1895) reported centrospheres in *Fossombronia, Aneura multifida* and *Fegatella conica* but was unable to find well-defined centrospheres in *Scapania undulata*. He observed a minute dense particle at the focus of the rays forming the centrospheres and stated that the existence of the centrosome was for him a secondary matter, since he regarded it as a mere insertion point, a granule or perhaps a condensation mass.

The account of Davis (1901) agreed with that of Farmer and Reeves, as does that of Chamberlain. However, Davis observed that the radiations of the asters were very irregular in distribution and variable in number, and that no centrosome was present, although the interior of the aster was frequently granular. He stated definitely that no centrosomes or asters accompanied the

resting nucleus. Gregoire and Berghs (1904) agreed with Davis and Chamberlain that no centriole or central corpuscle was present, and they felt that the centrospheres were accidental formations without any role or importance. Van Hook (1900) had reported that no centrospheres were present in the spermatogenous tissue of *Anthoceros*.

In 1905 Bolleter gave a brief description of the spermatogenesis of *Fegatella conica,* in which he found a centrosome at the last or diagonal division. He did not, however, find these centrosome-like bodies in the pre-diagonal divisions but concluded that they were present in the earlier divisions, since he saw them in the last division.

In 1906 Lewis found centrosome-like bodies at the poles of the spindles in the pre-diagonal as well as the diagonal divisions of the spermatogenous tissue of *Riccia natans,* but failed to find them in any other tissue. He did not regard these bodies as true centrosomes because they lacked genetic continuity, disappearing after each cell division but the last, where they persisted and functioned as blepharoplasts. He found no evidence of their nuclear origin and considered that they arose de novo with each division.

In his study of the spermatogenesis of *Fossombronia longiseta,* Humphrey (1906) noted the absence of the centrosomes, presence of the diagonal division, and the later appearance, in the cytoplasm of the androcyte, of a blepharoplast which soon migrated to the acute angle of the cell and became closely applied to the membrane. He further noted the appearance of the " chromatoider Nebenkorper " which he stated elongated and joined with the elongating nucleus, resembling, therefore, the " cytoplasmatischer Vorsatz " of Ikeno.

In 1906 Ikeno again pointed out that the centrosome in the liverworts plays a normal as well as a blepharoplastic function in some forms and is present only as a blepharoplast in other forms. This situation he held to have evolved in the course of the phylogeny of this plant group, when, according to Lamarckian principle, the body gradually disappeared because it no longer had a function. He cited Lewis and Humphrey and their respective reports on *Riccia* and *Fossombronia* as substantiating his own work on *Marchantia* and *Pellia.*

However, Ikeno admitted that the blepharoplasts of all plants

are not morphologically similar structures, as he was first inclined to believe. He accordingly proposed the following three categories: (*a*) centrosomic blepharoplasts, as were found in the myxomycetes, liverworts, vascular cryptogams and gymnosperms; (*b*) plasmodermal blepharoplasts, as in *Chara* and some Chlorophyceae; and (*c*) nuclear or karyo-blepharoplasts, found only in some flagellate groups.

Woodburn (1911), while studying spermatogenesis in *Porella,* found no evidence for the presence of centrosomes and concluded that because a body sometimes occupies the pole of a spindle, it does not indicate that it is any more a centrosome than are other bodies scattered through the cytoplasm. He stated that the blepharoplast develops de novo from kinoplasm located usually at the most distant angle of the androcyte and that it becomes a cord growing in close contact with the plasma membrane. He further thought that Ikeno's " cytoplasmatischer Vorsatz " was merely a part of the blepharoplast and he found nothing whatever corresponding to a " Nebenkorper ". In conclusion he felt that the blepharoplast and cilia represent specialized cytoplasm and that the remainder of the cytoplasm is in the vesicle. He also reported that there was no evidence for centrosomes in the spermatogenous tissues of *Porella* and *Asterella,* or in *Marchantia* and *Fegatella.*

Plants of groups other than the hepatics had also been used during this period for research on the centrosome problem. Ikeno investigated species of the moss genera *Pogonatum* and *Atrichum* and failed to find centrosomes. In 1911 Wilson reported that no centrosomes are present in *Mnium hornum* and *Atrichum undulatum* in the spermatogenous cells, but he noted the presence of blepharoplasts which he described as arising from the nucleolus. He also felt that centrospheres and probably centrosomes are present during the later divisions in the antheridium of *Pellia* and thought that the blepharoplast here is directly derived from a centrosome.

In 1900 Smith reported that no bodies subject to interpretation as centrospheres could be detected in the sporocytes of *Osmunda regalis.*

Davis (1908), in discussing zoospore formation in the alga *Derbesia,* stated that the nucleus in this organism has a very intimate relation to the blepharoplast and that the latter does not

arise from the plasma membrane, as Strasburger had noted for allied forms of *Oedogonium, Cladophora* and *Vaucheria,* but from granules associated with the protoplasm investing the nucleus. These granules, he held, are not centrosomes.

In the same year Yamanuchi (1908) stated that the centrosome is not observable anywhere in the life history of the fern *Nephrodium* and that the blepharoplast which arises de novo in the cytoplasm of the androcyte mother cell does not play the part of a centrosome.

The following year Chamberlain (1909) noted that the origin of the blepharoplast in *Dioon edule* was not easy to determine. He noted radiations from the blepharoplast and attributed its growth to the acquisition of granules.

In 1912 Sharp, noting that there were, at that time, two general views concerning the morphological nature of the blepharoplast as seen in the bryophytes, pteridophytes and gymnosperms, undertook the study of the spermatogenesis in *Equisetum* with the hope of shedding further light upon the relative merits of the two views. These views were: (*a*) the blepharoplast represents a centrosome; (*b*) the blepharoplast is specialized kinoplasmic or cytoplasmic material but is not a centrosome. His observations were that there are no centrosomes, centrospheres or asters in the early mitosis of the spermatogenous tissues of *Equisetum,* but that in the penultimate generation, a minute granule, surrounded by a weakly developed aster, appears in the cytoplasm in each cell. This granule subsequently divides into two, and each granule with its aster diverges to a different pole of the nucleus. A central spindle is formed and the nuclear membrane breaks down. These granules, now termed "blepharoplasts", occupy the poles, and, concomitant with the mitotic phases, they enlarge, become vacuolate and break up into a number of pieces. After further fragmentations these unite to form a cilia-bearing thread. On the basis of this research and upon consideration of his historical review, Sharp reiterated Ikeno's dictum that the blepharoplasts in the bryophytes, pteridophytes and gymnosperms are all homologous structures, ontogenetically or phylogenetically centrosomes. Sharp added that there could remain no question that this is so.

In 1912 Clapp was unable to find a centrosome during any division of the spermatogenous cells of *Aneura pinguis* and re-

ported that the blepharoplast arises in the cytoplasm of the androcyte.

Walker (1913) noted the presence of a centrosome-like body at each pole of the last division of the antheridial cells of *Polytrichum* and further noted that this body later functions as a blepharoplast. It was not possible to determine the origin of these centrosome-like bodies.

In 1913 Graham could not find centrosomes either in vegetative or in sporogenous divisions in *Preissia commutata.*

In *Riccia frostii* Black (1913) was unable to locate centrosome-like bodies in any of the spermatogenous divisions but noted the later appearance of a dense body in the androcyte which elongated and bore cilia.

In spermatogenesis in *Blasia pusilla,* Woodburn (1913) observed that the blepharoplast first makes its appearance as a dense area of cytoplasm in opposite ends of each of the pair of androcytes. These bodies gradually differentiate into a thread or chord near the plasma membrane. No centrosomes were found at any division, nor were there accessory bodies of any sort corresponding to the "Nebenkorper" of Ikeno or the "limosphere" of Wilson.

Apparently Sharp felt that his work on *Equisetum* was not definitive enough to answer all questions, for in 1914 he reported his observations on *Marsilia quadrifolia* in an attempt to clear up the points left in doubt by earlier workers. He added new details which he felt would make it possible to decide between the divergent views of earlier workers concerning the morphological nature of the blepharoplast. Sharp reported that the primary spermatogenous cell shows nothing which could be called a "centrosome". The many small granules which are evident at this stage appear to have no significance, and the spindle forms in the absence of any visible kinetic center. At metaphase the spindle ends rather indefinitely at the poles, in anaphase the regions appear denser, and subsequently distinct radiations emerge about each pole, where there is a dense and finely granular appearance. At telophase these polar achromatic structures disappear. The second mitosis proceeds like the first until anaphase, at which time the spindle becomes pointed and long radiations develop. At the center of these radiations there is a very minute and in-

tensely stained granule, the origin of which is impossible to make out. This granule, which Sharp referred to as the " centrosome ", now increases rapidly in size, and at last anaphase it is very conspicuous. At telophase the centrosomes are still enlarging and show indications of division. These centrosomes persist and are to be found in the cytoplasm of the resting cell, where they undergo division prior to the third mitosis. The two daughter centrosomes rarely diverge from one another, however, and subsequently degenerate. They are sometimes found at late prophase and metaphase of the third mitosis, but they bear no relation to the spindle poles which are at first rather indefinite, as in the first and second mitosis. The persistence of faint radiations about them helped to make their identification sure. During anaphase of the third mitosis they lie at the side of the spindle, and Sharp felt that they were non-functioning centrosomes which are to be identified with the " blepharoplastoid " of Shaw. At anaphase a new centrosome appears at each spindle pole exactly as in the second mitosis and behaves in a like manner. Division of these centrosomes occurs during telophase, and the daughter centrosomes diverge, showing a delicate spindle between them. The radiations on the side toward the nucleus become stronger and form two conspicuous rows of spindle fibers. At this point Sharp wished to call them " blepharoplasts " in view of the role which they are to play. The rays extending in other directions are not so well developed as in the third mitosis. The blepharoplasts now enlarge, develop one or more vacuoles and become irregular in outline. This process continues through the late anaphase of the fourth division, and at telophase they may be seen breaking up into several irregular pieces. The fragmentation continues until a considerable group of pieces has accumulated. These take the form of an irregular rod lying close to the nucleus or against it and bear the cilia.

Woodburn (1915) reported that the nuclear divisions in *Mnium affine* proceed in the usual manner without the accompaniment of polar bodies or plates and that the blepharoplast appears in the cytoplasm of the androcyte in which it functions. On the other hand, Allen (1917) stated, in substantiation of his work of 1912, that in *Polytrichum juniperinum* each newly formed androcyte contained a small rounded blepharoplast which behaved like a centrosome in the preceding division of the androcyte mother cell

and which, in most cases, still lay in the region just previously occupied by a spindle pole.

In 1918 Graham, who had previously (1913) reported the absence of centrosomes from *Preissia commutata,* observed centrosomes not only in the spermatogenous divisions and as blepharoplasts in the androcyte of *Preissia quadrata* but also in the fertilized egg at the time the pronuclei are paired. In addition, she stated that centrosomes and asters are readily demonstrable in the sporophyte.

In 1920 Sharp reported that the centrosome is found in all phases of the last antheridial division of the liverwort *Blasia pusilla* and that it then functions as a blepharoplast. This account disagrees with that of Woodburn (1913) who saw the blepharoplast developing in the androcyte. Sharp further disagrees with Woodburn in respect to the development of the blepharoplast into the flagellar apparatus. Whereas Woodburn noted a simple elongation, Sharp claimed that the blepharoplast breaks into several pieces which ultimately coalesce to form a short lumpy rod. He noted that this is the only known instance of fragmentation in the bryophytes and pointed out that fragmentation is a general characteristic of the cycads but occurs only occasionally in the pteridophytes (*Equisetum* and *Marsilia*). Therefore, he held that it is evident that fragmentation occurs in forms lower in the scale. He pointed out, furthermore, that the fission of the blepharoplast of *Blasia* and the more complex fragmentation of the blepharoplasts of *Equisetum, Marsilia* and the cycads may be homologized with the normal division exhibited by ordinary centrosomes.

In summing up, Sharp presented the conclusions which are the generalizations commonly accepted to be true at the present time. They are: (*a*) the blepharoplasts of bryophytes, pteridophytes and gymnosperms are ontogenetically and phylogenetically centrosomes; (*b*) these centrosomes become more and more restricted in the life history in passing upward through these groups; (*c*) they are retained in the spermatogenous cells because of the cilia-bearing function which they perform there; and (*d*) in connection with this latter function, they have become profoundly modified, losing many of the characteristics of centrosomes and assuming new characteristics not exhibited by centrosomes elsewhere.

A careful study of various stages of mitotic divisions in *Asterella*

convinced Woodburn (1922) that polar caps and granular bodies arise de novo as the nucleus enters upon division and that there was no basis for assigning to them morphological rank any more than to the fibers of the spindle. Furthermore, he stated that, while polar granules occur irregularly, no bodies are evident which partake clearly of the nature of centrosomes. He noted that similar granules may occur with irregularity in number and position throughout the cytoplasm.

In an account of spermatogenesis in *Anthoceros laevis,* Bagchee (1924) stated that the centrosome is entirely absent from *Anthoceros* and that the blepharoplast which arises in the androcyte appears to arise by a fragmentation from the main mass of chromatin of the nucleus.

Bowen (1927) felt that, in general, plant cells are characterized by the absence of central bodies but noted that central bodies are often called " blepharoplasts " by botanists. He insisted that in a plant cell, if a centriole-like body is related to a vibratile filament, the conclusion that this body is a true centriole would appear unavoidable. His work with *Polytrichum* supported the conclusions of Wilson (1911) and opposed those of Allen (1917), and he believed his observations left no doubt that the body to which filaments are attached is a true centriole comparable in every way to the central bodies of animal cells.

The blepharoplast in *Plagiochila adiantoides,* as reported by Johnson (1929), first becomes clearly evident as a short rod when the nucleus of the young spermatozoid begins to elongate. It can not be found consistently at the poles of the last mitotic spindle and it is never seen in earlier mitosis. No clear case of vacuolization and fragmentation of the blepharoplast can be found.

In 1931 Yazawa could not find centrosomes in *Makinoa crispata* either in early division of the spermatogenous cells or in the divisions of the androcyte mother cells. In the androcyte the blepharoplast seems to arise de novo in the cytoplasm near the pointed end of the cell. Yazawa pointed out that this is in marked contrast to the condition existing in *Marchantia polymorpha,* where, he stated, definite centrosomes are present at the spindle poles in the last antheridial division.

In her work on the spermatogenesis of *Bucegia romanica,* Eftimin (1933) reported that centrosomes appear only in the last

division of the antheridial cells and that in the androcyte there is a blepharoplast whose origin seems to be centrosomal.

In a related genus, *Dumortiera hirsuta,* no centrosomes could be seen by Patterson (1933) who stated that the blepharoplast makes its first appearance in the androcyte.

In 1934 Rankin noted that the blepharoplasts of *Polypodium polypodioides* arise in the resting stage of the spermagones before the last nuclear division in the antheridium, where they occupy the poles during the subsequent mitosis. When the androcytes are formed, one blepharoplast is in each cell and is situated at some distance from the nucleus.

Feng (1932a, b, 1934), the most recent proponent for the presence of centrosomes in the angiosperms, presented evidence from his researches on the *Caprifoliaceae,* but Eichhorn (1933), Gavaudan and Yu Chich-Chen (1936), and the author (unpublished) showed this to be in error.

Marengo (1949), in his work on the cytoplasmic inclusions during sporogenesis in *Onoclea sensibilis,* could not find centrosomes but mentioned that the concentration of cytoplasmic granules at the polar terminations of the spindles of the first meiotic division is suggestive of centrosomes in animal cells.

Lang (1936), in an investigation concerned mainly with the structure of the mature spermatozoid, indicated that the blepharoplast structure is composed of two identical and parallel strands of material similar to nucleolin of the nucleolus and that the cilia-bearing band, closely associated with the blepharoplast, consists of plastin, another nucleolar constituent. He further pointed out that the cilia do not arise from the blepharoplast but are outgrowths of a special band which is in contact with the blepharoplast, as Muhldorf had pointed out in 1930. However, he was disposed to add that it seemed clear that the blepharoplasts of the thallophytes, bryophytes, pteridophytes and gymnosperms are all strictly homologous structures and that it likewise appears obvious that blepharoplasts, in many instances, are ontogenetically derived from centrosomal material. These deductions, he confessed, revealed nothing of the real nature of the blepharoplast. He listed the outstanding views regarding the nature of the blepharoplast current at that time: (*a*) the blepharoplast, a centrosome-like body, is an organ sui generis and develops in the cytoplasm during the

spermatogenous divisions (the blepharoplasts are cytoplasmic organs), (*b*) the blepharoplasts represent chondriosomal material of the androcyte, as has been advocated by Motte and Dracinschi; and (*c*) the blepharoplast substance finds its ontogenetic origin specifically in the nucleolus.

Yuasa (1939), in his observations on the origin of the blepharoplasts and centrosomes in plants, sought to point out the similarities between the blepharoplast and the nucleolus on the basis of physico-chemical properties and staining reactions. He further pointed out that the various researches, including Ikeno's (1903), seem to indicate that the blepharoplast is derived from the centrosome which in turn originates in the nucleus, but he admitted that direct evidence for this latter phenomenon has not been found. On the basis of his studies, Yuasa felt that the double structure theory of the blepharoplast and the centrosome is correct. He further stated that all the considerations of the nature of the blepharoplast could be included in two categories: (*a*) the blepharoplast is derived from the nucleus, and (*b*) the blepharoplast is derived from the cytoplasm or the cytosomes. He was inclined to believe that the homologous relationship between the cytoplasm and the blepharoplast is precluded on the basis of staining reactions and physicochemical properties.

From a consideration of this literature has arisen the general acceptance that the centrosome is present in the plant kingdom, where it is supposedly restricted to those plants which still retain motile gametes. It should be pointed out again that even in these cases the centrosome per se is not always present but that those who feel that the blepharoplast is derived from the centrosome, use these terms interchangeably.

There seems never to have been any serious dispute about the presence of true centrosomes in many of the algae and fungi (Strasburger et al., 1897; Trow, 1904; Wolfe, 1904; Bagchee, 1925; Fritsch, 1935, 1945). However, the origin of the blepharoplast in at least some of the algae is held to be plasmodermal rather than centrosomic (Davis, 1904a, b, 1908; Mottier, 1904).

A survey of the centrosome problem in the algae indicates that centrosomes or centrospheres have been reported in all the major groups but in relatively few genera (Fritsch, 1935, 1945). The Phaeophyceae appear to offer the clearest examples of centrosomes.

These have been most readily demonstrable in the higher orders, and all gradations have been reported from clearly marked, deeply staining granules to such as are indicated only by pronounced radiations at the spindle poles (Fritsch, 1935; Smith, 1938). The continuity of these structures through successive cell generations has not been demonstrated, and there is some doubt as to their mode of origin (Yamanouchi, 1908).

In the Chlorophyta, blepharoplasts have been reported by Mottier (1904), Davis (1904b) and others, but no connection with a centrosome-like body has been observed. On the other hand, a complex neuromotor apparatus in *Chlamydomonas* is reported to arise from an intranuclear centrosome (Smith, 1938). Thus there is considerable variation with respect to the presence of centrosomes and blepharoplasts attributed to this group from which it is held our land plants arose. Possibly there is a solution in this group to the problem of the origin of the blepharoplast.

In the Hepaticae and the Musci, the presence of the blepharoplast in the androcyte [2] and the absence of centrosomes from all other cells except spermatogenous ones are explained by the allegation that, in the evolution of these plants, the centrosome has become restricted to the spermatogenous division, where it finally functions as a blepharoplast. In instances where there is only a blepharoplast, it is pointed out that the centrosome has reached the ultimate in its modification and now appears and functions only in the androcyte in the formation of the flagella or cilia, as the case may be.

Therefore the statement that the blepharoplast is phylogenetically and ontogenetically a centrosome, first uttered by Belajeff, echoed by Ikeno, and re-echoed by Sharp, is today accepted as fact (Yuasa, 1939; Lang, 1936). This in spite of the fact that much of the evidence upon which the postulate is based has been placed in doubt by the contrary evidence of a multitude of investigators. The failure of some investigators to find centrosomes in materials

[2] The term "androcyte" (Allen, 1912) is used for the cells produced in the last division of the antheridium, which cells are destined to be metamorphosed into the sperm. The cell from which the androcytes originate is designated the "androcyte mother cell". The term "spermatid" is not so appropriate as "androcyte", since the former is also used for the cell generation in metazoa which arises directly from the meiotic process. The male gametes are referred to as "sperms".

in which centrosomes have previously been reported, and the agreement that not all blepharoplasts are of centrosomic origin, as is indicated by the classification of Ikeno, Chamberlain, Wilson and Yuasa, cast definite doubt upon the validity of the aforementioned generalizations.

General Critique

Ever since the centrosome was discovered in animal cells, controversies have existed relative to the extent of its occurrence, its reality (Hughes, 1952) and its homology with the blepharoplast in the androcyte of the plant cell (Belajeff, 1897; Ikeno, 1903). More recently the problem has centered around the determination of its chemical nature and its ontogenetic origin where it appears in only one or a few cell generations (Lang, 1936; Yuasa, 1939).

The early conclusions of Van Beneden and Boveri (Wilson, 1928) that the central body, like the nucleus, is a permanent and autonomous component of the cell were soon assailed with the early discovery that these structures are not present in the cells of many of the plants in the groups above the thallophytes. Furthermore there was the question, on the basis of cytological and experimental evidence, of whether or not these bodies can be formed de novo in the cells of certain animals (Yatsu, 1905).

The early literature is replete with synonyms for what has been commonly regarded as the centrosome. "Centrosphere", "centralbody", "centriole", "centroplasm", "center", "directivesphere", "division-center", "microcentrium", "astrosphere" and "periplast" are a few of the terms applied to the polar structures composed of either a deeply staining granule or a more or less spherical hyaline area surrounded by rays or some combination of the two. In some instances astral rays alone led investigators to postulate that a centrosomal structure must be present. The morphological aspect most frequently used and perhaps most descriptive of the concept of a centrosome held by present-day biologists is that of a deeply staining granule at the spindle pole with or without a surrounding non-stainable area and usually located at the focal point of numerous astral rays.

Wilson (1928) noted that the word "centrosome" was found in the literature in at least four senses, and further noted that, at

the very outset, Boveri's concept and that of Van Beneden were different. To avoid this ambiguity of terms, Flemming and others adopted the more inclusive and non-committal term "central body", "division-center" or simply "center" in all cases where the genesis of the central apparatus was not fully known. It can not be denied that this device helped to bring about some consistency in the subsequent reports, but, at the same time, there have been reported, under a single name, all variations of this theme, thus giving only apparent consistency to the reports. Perusal of the literature indicates that this terminology was not universally adopted. The term "centrosome" appeared to be extended, especially by the botanists, to mean essentially what the term "central body" implied. Cleveland (1953) has recently stated that the terms "centrosome" and "centriole" can not be used as synonyms and that the achromatic figure is always produced by centrioles—never by centrosomes.

Furthermore, as the term "blepharoplast" was used more extensively, and the tendency to homologize it with the centrosome grew, more frequent reference was made to the "true" centrosome, to distinguish it from the blepharoplast which was held to be centrosome-like when it arose in the cell generation prior to the one in which it was to carry out its function. As has been previously mentioned, questions arose, from time to time, as to the reality of the centrosome in any cell (Hughes, 1952), but the reports of the presence of centrosomes in living tissue (Huettner and Rabinowitz, 1933; Cleveland, 1935a) has answered this argument.

That there has been no retreat from the original concept of the function of the centrosome is attested to by Darlington's (1949) description of the centrosome as a self-propagating spindle-organizer in animals and lower plants.

The absence of centrosomes from the anastral spindles of most plants has presented a problem not yet adequately explained. However, the work of Pollister (1943) on snails, which indicates the possibility that the centrosome had its phylogenetic origin in the disembodied centromere, would lend credence to the theory that the chromosomes through the medium of the centromere are capable of organizing the spindle where centrosomes are absent.

The relationship of the blepharoplast to the centrosome in plants

has posed another problem which, to all intents and purposes, was solved, at least to the satisfaction of the majority of biologists, by a series of works extending from 1898 to 1914. The most important of these were the reports of Belajeff (1899), Shaw (1898), Webber (1901), Ikeno (1903) and Sharp (1912, 1914). Naturally the evidence produced by other workers in this period was not unimportant, for some of it gave credence to and tended to substantiate the work and speculations of those men who furnished the milestones of what is now popularly held to be the answer to the question of the centrosome-blepharoplast homology. Wilson (1928), in his treatment of the literature on this problem, cited Belajeff (1899), Shaw (1898) and Webber (1901) as having traced the blepharoplast beyond a doubt to a position at or near the spindle poles of the last division. In mentioning that many botanists have been reluctant to recognize the homology, Wilson cited the objections of Webber as they are presented earlier in this paper. However, he noted that the force of these objections was much weakened with the work of Sharp (1912, 1914), which was foreshadowed by Shaw (1898) and Belajeff (1899). Ikeno (1903) was cited as filling in the gap for the Hepaticae, and Allen (1912) for the Musci. Therefore Wilson felt that in view of the facts, it was impossible to doubt the correctness of the view, earlier urged, especially by Belajeff and Ikeno, that the blepharoplasts of the higher plants are morphologically and physiologically similar to those of animal sperms. The only problem that could arise, Wilson continued, related to the nature of the central body itself and its proper definition in the light of these facts.

To the present writer, however, there appears yet another problem that must be solved if the centrosome-blepharoplast homology is to be placed on a firm foundation. That problem stems from the nature of the evidence upon which this speculation is based. First of all, it would appear necessary to determine the validity of the evidence relative to the actual structures observed; secondly, to determine the validity of the interpretation placed upon it. Naturally much of this deliberation must be based upon a consideration of the work of the aforementioned researchers.

It is extremely difficult to evaluate the reports of the many researchers who have been concerned with the centrosome problem in plants. Some of the difficulty stems from the fact that the

terminology is not precise, some from the fact that the usual techniques do not always give the same results in different hands, and perhaps some from the vagaries arising out of different interpretations. One outstanding example of such difficulty is found in the work of Graham. In 1913 Graham studied the nuclear division of *Preissia commutata* and was unable to find centrosomes or centrospheres in any of the somatic or heterotypic divisions. In describing her work (Graham, 1918) on the fertilization stages of *Preissia quadrata,* however, she stated that it was evident that distinct, granular centrosomes are present, not only in the divisions just preceding spermatogenesis and as blepharoplast during metamorphosis, but also in the fertilized egg at the time the pronuclei are paired. She added that centrosomes and asters are readily demonstrable in the sporophyte of *P. quadrata.* Ordinarily there would be little to consider unusual here, since these researches were conducted with different species. But in 1926 a report by Haupt, dealing with the morphology of *Preissia quadrata,* provided enlightening information. Haupt said that *Preissia quadrata* (Scop.) Nees was the name adopted for this liverwort by Evans, and that *Preissia commutata,* the name used by various writers for the same species, was relegated to synonomy. Now the question arises, did Graham actually get different results with the same species, or was she dealing, in each case, with a different species? To answer this is a problem in itself, the solution of which would require intensive research dealing with taxonomic decisions as well as cytological aspects. In relation to the overall problem, it is, perhaps, not worth the time and effort necessary, particularly when no similar evidence for the presence of the centrosome has been produced in recent years (Showalter, 1926b, 1927a, b).

As has previously been suggested, speculation can certainly be no more valid than the facts upon which it is based. One of the liverworts, *Marchantia,* plays a very important role in the speculation of all the investigators writing on this problem since Ikeno (1903) first reported the results of his investigations.

As Gavaudan (1930) has noted, *Marchantia polymorpha* provides some distinct advantages for the investigator of spermatogenesis. Historically it has been utilized for this type of research by many investigators, and, therefore, a considerable bibliography exists on the spermatogenesis of this organism. Much of the

literature deals with the presence of the centrosome or its absence. But in spite of the many advantages of prominent easily handled antheridia, in which many cells in the same stage of division may be found and the easily determined last division, there exists the distinct and important disadvantage of extremely small size. The limitations imposed upon the investigator by this small cell size are considerable. Even the most casual observation of this tissue requires high magnification, and a good light source is indispensable. It was based on research in this organism that Ikeno (1903) indicated that the centrosome is present in the spermatogenous cells of the bryophytes, thus enabling him to reinforce Belajeff's dictum regarding the centrosome-blepharoplast homology. This was the first detailed description of spermatogenesis in the Bryophyta (Wilson, 1911).

Previous to Ikeno's work, the centrosome had been reported in the vegetative cells of *Marchantia* (Van Hook, 1900; Mottier, 1898), but subsequent investigations showed this to be erroneous. Of the later investigations, Ikeno (1903) and Schaffner (1908) were the only ones, to this writer's knowledge, who reported the centrosomes to be present in all antheridial divisions. On the other hand, Miyake (1905), Escoyez (1907), Woodburn (1911, 1913), Gavaudan (1930), Yazawa (1933) and others have denied the presence of centrosomes in the earlier divisions but have observed polar corpuscles in the last or diagonal division. Miyake and Escoyez and Woodburn were inclined to look upon these bodies, the blepharoplasts, as organs which are separate and distinct from the centrosome, but Gavaudan (1930) and Yazawa (1931) felt that the centrosome homology is a valid one. Elsewhere in the Marchantiales is conflicting evidence. In *Conocephalum* (*Fegatella*), for example, Bolleter observed bodies in the last division, and on the basis of this observation he concluded that they are present in the earlier divisions. Needless to say, this is not very convincing evidence for the presence of centrosomes in the earlier divisions. This work has often been quoted, however, to support the idea that the centrosome and blepharoplast are homologous. Escoyez (1908), Miyake (1905) and Woodburn (1911) could not find the centrosome in the early divisions of the spermatogenous tissue of *Conocephalum*.

In several related genera, *Asterella* (Woodburn, 1911) and

Dumortiera (Patterson, 1933), the blepharoplast first appears in the androcyte, while in *Bucegia* (Eftimin, 1933), it is found in the androcyte mother cell.

In *Riccia* Lewis (1906) reported centrosome-like bodies at the spindle poles of all of the antheridial divisions, including the last, where this body allegedly functions in the production of the flagella. However, Lewis felt that, since this body apparently arises de novo in the cytoplasm at every division, it lacked genetic continuity and could not be called a "centrosome". Although Lewis figured this structure prominently in his plates, the photographs which accompanied them to substantiate the figures, cause an opposite impression. No one would doubt that these were the author's best photographs of his most definitive preparations. However, this writer feels that Lewis did not find centrosomes in his material, and that what he photographed could not even be accurately described as centrosome-like, difficulties of photographic reproduction notwithstanding. Black (1913) was unable to find centrosomes or centrosomelike bodies in any of the spermatogenous divisions of another species of *Riccia,* where she reported the blepharoplast as arising in the androcyte mother cell.

All investigators have reported the absence of centrosomes from the early antheridial divisions of *Conocephalum* (Miyake, 1905; Escoyez, 1908; Woodburn, 1911; Lepper, 1954), except Bolleter (1905) who did not study these divisions but assumed the presence of centrosomes in them because he had found centrosomes during the final division. In essence the writers are in agreement that a granule is sometimes present during or at the completion of the last division.

Farmer (1895) had reported centrospheres in the first division of the spore mother cell of *Conocephalum,* in material fixed with alcohol. His observation that possibly a third centrosphere is present appears to be indicative of fixation artifacts rather than the presence of actual morphological entities. Moreover, the so-called centrosphere, although considered by some to be a manifestation of centrosomal activity, lacks the essential structure—the centriole.

In this genus one often finds dark-stained granules near or on the spindles which are rather prominent. Frequently they are found on either side of the equatorial plate. In the telophase

these granules may be seen alongside the mitotic figure or on the phragmoplast. Gavaudan (1930) described similar structures in *Marchantia* and held them to be plastids. In Feulgen preparations these granules can not be seen and, therefore, are considered to be Feulgen-negative, as would be expected for plastids.

It might be pointed out that an illustration by Woodburn (1911, Plate XXV, fig. 53), purporting to show bodies at the diagonal spindle poles, also has two identical bodies on opposite sides of the metaphase figure and in the vicinity of the equatorial plate. Perhaps these are plastids. Escoyez (1907, Plate I, fig. 25) gives a somewhat similar impression for the diagonal division of *Marchantia.*

If we consider for the moment only, those liverworts which are related in the order Marchantiales, we find that there are no undisputed accounts wherein the centrosome is reported to exist in the form of a polar granule in all of the spermatogenous divisions. Actually there have been only two species in two genera of this group, *Marchantia polymorpha* and *Riccia natans,* in which centrosomes or centrosome-like bodies have been described as existing throughout these divisions. As was indicated above, the evidence for this condition does not seem to be valid. In *Marchantia* their presence was disputed by several investigators prior to 1913, but, nevertheless, Ikeno's observations have been quoted almost in their entirety, and only the origin of the centrosome from the nucleus has been questioned. The origin was not doubted in the third edition of Sharp's cytology text (Sharp, 1934), nor in E. B. Wilson's third edition (Wilson, 1928). In 1930 Gavaudan was unable to show centrosomes in the earlier divisions and reported the blepharoplast as first seen in the last division.

The writer's observations (Lepper, 1954) on the spermatogenous tissues of *Marchantia* have indicated to him that the centrosome does not exist in these divisions, nor could he find an undoubted blepharoplast in any of the division stages leading to the formation of the androcyte.

The blepharoplast has been observed in the diagonal division in so many instances of previous investigations, that one can not doubt the possibility that the investigators were, on the whole, correct in their observations, although Escoyez's drawings leave

some doubt in the present author's mind that the bodies he figured were truly blepharoplasts.

According to Gavaudan's treatment of the subject (1930), Motte was cited as having claimed not to be able to find the blepharoplasts in the diagonal division of the antheridial cell. Woodburn (1911), although he noted polar corpuscles during the diagonal division, claimed that they did not persist very long after the metaphase stage and he reported that the blepharoplast arises de novo from an aggregation of cytoplasmic granules in the acute angle of the androcyte. In some cases in the Hepaticae, where the blepharoplast is reputed to be of centrosomal origin (Ikeno, 1903; Schaffner, 1908), the blepharoplast is reported to appear in the androcyte in the same area where the spindle pole was located in the previous division. It is reported that it then moves to the acute angle of the androcyte. Approximations of size and location of granules held to be similar in these cases, leave much to be desired in the way of convincing evidence. However, both Ikeno (1903) and Schaffner (1908) included in their figures of *Marchantia* a continuous series of stages from the androcyte mother cell to the developing androcyte, showing the dense granule which they held to be the centrosome.

The figures of both Schaffner (1908) and Ikeno (1903) are not particularly convincing to one who has viewed sectioned material of this same organism. Ikeno's (1903b) figures 32-33, depicting so clearly the development of the flagella from the blepharoplast in sectioned material, causes one to wonder whether perhaps a certain amount of artistic license and a devotion to a preconceived idea might not have played a part in their composition.

In other genera of the Hepaticae similar discrepancies in the reports are noted.

The earliest investigations of *Pellia* with reference to the centrosome problem were conducted by Farmer and Reeves (1894) who described centrospheres in the early divisions of the germinating spores. These could not be demonstrated in the resting stage, and, although a particle could be distinguished at the center in some instances, it was not possible for these authors to ascertain the existence of a centrosome. They noted that it is easy to find bodies of minute size surrounded by diffraction areas but felt it is pure empiricism to select one or two of these and call them

"centrosomes". This material was alcohol-fixed. In a later paper in which he investigated several hepatics, Farmer (1895) reiterated what had been observed in *Pellia* in the original investigation.

As a result of his researches on *Pellia,* Davis (1901) reported that there are definitely no asters or centrosomes accompanying the resting nucleus, nor are these structures to be found in the divisions differentiating the spores. However, he reported that centrospheres with radiations resembling asters occur in the first two or three mitoses of the germinating spore. He spoke of their developing at prophase, disappearing at metaphase and finally giving way to polar caps in the more mature phases of the gametophyte. In the vegetative divisions of the setae, no centrospheres are present.

Chamberlain (1903) confirmed Davis's observations on the germinating spores and added that no centrosome or centrospheres are found during the mitoses in the apical region of the thallus or in the developing antheridia. It was not determined whether a blepharoplast occurs toward the close of spermatogenesis.

Miyake (1905) likewise was unable to find centrosomes in the dividing antheridial cells.

Wilson's observation (1911) on the antheridial divisions agreed with Chamberlain's up to the stage where the antheridia showed 30 or more cells in transverse section. However, he found centrospheres similar to those described by Farmer and Reeves in the germinating spores, but the cytoplasmic radiations were not so numerous. Occasionally a small granule was to be seen at the center of the radiations, but the presence of a centrosome was held to be doubtful. At the metaphase Wilson could not find radiations with certainty, although he mentioned that here again a small granule is occasionally seen at each pole. In the last division Wilson again mentioned the occasional occurrence of small deeply staining granules in the vicinity of the poles. The blepharoplast arises in the androcyte, and, though Wilson was not able to determine its origin, he maintained that the position in which it is first observed is suggestive, since this spot was occupied by the pole of the spindle in the division of the androcyte mother cell. This is the place where a centrosome, if present, would probably be found. It is curious to note that on the basis of this evidence,

Wilson concluded in his summary that, in *Pellia epiphylla,* centrospheres and probably centrosomes are present during the later divisions in the antheridium and that the blepharoplast is probably derived directly from the centrosome. The present writer did not see the article by J. and W. van Leeuwen-Reijnvaan (1908), but, according to Wilson's citation of this work, these authors claimed that a single centrosome is first present in the cytoplasm of the spermatogenic cell of *Pellia* and that this, by division, produces the two centrosomes found later at the spindle poles. Wilson could not confirm this.

Gregoire and Berghs (1904) felt that the centrospheres must be accepted as accidental formations without function or importance. They suggested that the centrospheres observed by Davis and Chamberlain are not organs sui generis, as these observers had thought, but merely the junction of astral threads.

It might also be mentioned again that at this time considerable confusion existed relative to the precise definition of the terms " centrosome " and " centrosphere ", as well as many other terms such as " centriole ", " attraction sphere ", " polar corpuscles " and " astrosphere ". Essentially, Farmer's " centrosphere " appeared to refer to distinct bodies whose composition was much like that of the astral rays which sometimes surrounded them. A granule or dense portion within the centrosphere was designated as the " centrosome ".

It is this writer's observation (Lepper, 1954) that there are no centrosomes or centrospheres in the antheridial tissues of *Pellia epiphylla,* either in the early divisions or the last division. The blepharoplast arises de novo in the later stages of the androcyte and is located in the cytoplasm in the acute angle of the androcyte.

There have been few studies of the mitoses of *Pallavicinia.* Farmer (1894) reported centrospheres but no centrosomes and noted that, in this respect, *Pallavicinia* was similar to *Aneura multifida.* Moore (1903, 1905) pointed out that sporogenesis in *Pallavicinia* was essentially in agreement with that of *Pellia,* as reported by Davis (1901), and that there was no evidence whatever for the existence of centrospheres, centrosomes or astral rays. The author (Lepper, 1954) was unable to find centrosomes or centrospheres in any of the antheridial divisions.

Both Van Hook (1900) and Davis (1899) had reported that

centrospheres were lacking in the sporogenous tissue of *Anthoceros*, but no further work relative to this problem seems to have been done in *Anthoceros* until Bagchee (1924) made his investigations on the spermatogenous tissue. In a rather detailed study, Bagchee reported that the centrosome is entirely absent from *Anthoceros*. He further reported that the blepharoplast arises in the androcyte as a result of the fragmentation of the main mass of chromatin, and that it lies for a short time at one end of a narrow strip of chromatin. The blepharoplast disappears as soon as cilia are visible around the young sperms.

Lepper (1954) was able to confirm some of Bagchee's observations but regards others as without sufficient basis in fact. The metaphase spindles are as Bagchee described them, sharply pointed and devoid of centrosomes. The final division is diagonal but irregularly so, as Bagchee had also noted. The mitotic figures of *Anthoceros* are rivalled only by *Marchantia* for smallness. It seems altogether problematical that Bagchee could have determined the nuclear origin of the blepharoplast and have noted the origin of the cilia. In fact, it appears to this observer that the blepharoplast was not seen by Bagchee. He drew a granule supposedly representing the blepharoplast, but the size, compared to the nucleus, seems unreasonably large, when the relative size of the blepharoplast in other bryophytes are considered. What is more, the present writer is convinced that the cilia of this organism can not be put to evidence by the ordinary staining methods used by Bagchee. The notion that the blepharoplast of *Anthoceros* arises through fragmentation of the main mass of chromatin seems to be based on evidence presented by Digby (1909) to whose work Bagchee made reference. However, even though the work of Digby on nuclear extrusions in *Galtonia* can be extended to the situation obtaining in *Anthoceros*, it does not follow that any of the extrusions are destined to become functional blepharoplasts.

It should be pointed out that in almost all instances, the researchers dealing with centrosomes have tried a variety of fixatives and stains, and inevitably they have obtained their best preparation from material fixed in Flemming's fluid and stained with iron-alum hematoxylin. The writer has found this combination to give good results in putting the mitotic figure in evidence. Both picro-formol and chrom-acetic have yielded good results also, but the spindle

elements are not so well-defined, nor are the spindle fibers so clearly delineated. It appears, further, that many of the discrepancies in the accounts of the observers are not the result of different fixations or staining methods, or even technical ability differences, but lie in the interpretation of the observations.

Sharp (1912), in his paper on *Equisetum,* agreed with Belajeff's conjecture of the homology of centrosomes and blepharoplasts (1898), and he further pointed out the similarities of the centrosome and the blepharoplast. In his paper on *Marsilia,* Sharp (1914) repeated Shaw's (1898) and Belajeff's (1899) work to show the appearance of a body resembling a centrosome in all but the first of the four spermatogenous divisions. These observations were instrumental in convincing biologists of the veracity of the centrosome-blepharoplast homology. It is, therefore, necessary that these works be examined with reference to the actual evidence and the possibility of alternative speculation based upon this evidence.

Sharp noted that, in *Equisetum,* it is necessary to select for study those antheridia in which the plastids do not introduce an element of uncertainty, since these cells may contain plastids in considerable number and in various stages of disorganization. It would appear that even the clearest of these cells might contain some granular elements of plastid disorganization, as is perhaps evident in Sharp's fig. 5. The difficulty of distinguishing between discrete granules in the cytoplasm and polar bodies is inherent in nearly all researches on the plant centrosome. The granules which Sharp first took to be the blepharoplasts had a diameter of 0.25 to 0.3 micron, with very faint radiations. He submitted evidence of their division in successive mitotic figures showing similar pairs of granules at varying distances around the circumference of the nucleus. In a figure such as Sharp's fig. 5, which he held to be an exceptional cell, he showed many bodies similar to those he interpreted as blepharoplasts. He claimed, however, that the real blepharoplasts could be distinguished from these granules in that the blepharoplasts were larger than any other pair in the cell, that they were always nearest the nuclear membrane and that they had the most evident radiations. This last distinction would seem to imply that the others also had radiations. He admitted that one could hardly speak conclusively regarding all points in the history

of such minute structures. This evidence, however, inclined Sharp toward the belief that the original granule, which by division gave rise to the two blepharoplasts, is, in some cases, one of the number which appear de novo in the cytoplasm and begin development.

Sharp reported that as the two blepharoplasts move apart, a very distinct central spindle is seen, so that a faint but undoubted amphiaster develops between them. During the early stages of the migration, this central spindle gradually fades out. Examination of Sharp's figures causes this writer to doubt that there ever is a true amphiaster, distinct or otherwise. Moreover, a structure that might have been considered an amphiaster could have been merely the intermeshing of artifactual rays surrounding two granules, thereby giving the effect of a spindle. Where other granules were observed at a greater distance apart, there would be little or no intermeshing of these rays, and thus an apparent gradual disappearance of the spindle thought to have been previously formed. That the fibers from the blepharoplast extend into the nucleus and establish the karyokinetic figure, as reported by Sharp, is the purest of speculation. The point that Sharp wished to make was that in *Equisetum* the centrosomal character of the blepharoplast is demonstrated by the production of an amphiaster. This would be perhaps unmistakable evidence of a centrosomal nature if it is true, but the evidence does not appear convincing to the writer.

Belajeff first homologized the blepharoplast of *Equisetum* with the centriole of animals because, since the blepharoplast gives rise to the cilia, it is similar to the centrosome of the animal spermatid, which also gives rise to the middle piece and filament of the sperm.

Sharp's investigation of *Marsilia* (1914) to a considerable extent verified the observations of Belajeff (1899) and Shaw (1898) on this same organism. Sharp disputed Belajeff's opinion that the body at the spindle poles has genetic continuity. He agreed with Shaw that these bodies arise de novo with each division and that they degenerate in the cytoplasm in all but the last division. There they persist and divide and form the achromatic figure for the last division. Sharp failed to see these bodies at the metaphase of the third division, as Shaw and Belajeff did, and claimed that they always arise during anaphase. However, Sharp felt that if he had looked further, he would probably also have found this

stage. Here, as in *Equisetum,* the evidence for formation of the spindle from the apparently diverging centrosomes is not very convincing. His fig. 29 and 30 were similar to those he presented for *Equisetum,* and the same argument will serve to suggest their lack of reality.

In his fig. 31, depicting the appearance of the cross-section of a developing androcyte of *Marsilia,* there is shown what Sharp held to be starch and other disorganized material in the cytoplasm. This material is shown to be darkly stained, which suggest that there is no reason why the granules in the cytoplasm that were held to be centrosomes could not have been of this same nature, unless it be the presence of the faint asters which surround them. However, Kuwada and Maeda (1929) have presented evidence that the astral rays around the blepharoplasts of *Cycas revoluta* are fixation artifacts and that they are entirely lacking in the living cell. This, together with the fact that cytasters can, under abnormal condition, be produced in the cytoplasm (Yatsu, 1905) would suggest that the presence of asters would be one of the least impressive bits of evidence, unless they are at the spindle pole where they would merely serve as a guide for the location of a small body at that point. Even as early as 1899, Fischer, as cited by Hughes (1952), showed that when osmic acid is added to elder pith impregnated with protein, progressive formation of an aster results around the remains of the nucleus. What is more, it seems extremely strange that a spindle would be formed at the end of hundreds of divisions, through the intervention of a central body which had arisen, in turn, at the spindle poles of the previous mitosis. It should also be remembered that Shaw (1898) reported that these bodies arise near the spindle poles, and not at them, as Sharp (1914) and Belajeff (1899) reported. Furthermore, the obvious orientation of the cytoplasmic structure might well determine the position of discrete bodies in the cytoplasm or perhaps determine the physiological centers giving rise to these bodies.

It is necessary also to consider the evidence for the presence of centrosomes in the mosses. Here the work of Allen (1911, 1912) on *Polytrichum* has been cited as showing a distinctive relationship between the centrosome and the blepharoplast. The cogent feature of this work is a body that appears in the androcyte mother cell surrounded by a few rays. This body subsequently divides and

appears at the poles of the division figure which give rise to the androcytes. In the androcyte a body is discovered, usually in a position corresponding to that of the central body in the previous divisions, but sometimes in the center of a system consisting of a few radiations. These radiations are similar to those surrounding the body which is found previous to the initiation of mitosis.

The evidence relating to the centrosome problem presented by Allen seems to be plausible. Yet there are some features which are difficult to reconcile with the action of centrosomes as they are known in animal cells. For instance, Allen reported that the prophase nucleus swells and comes in contact with the polar bodies, whereupon these bodies are much less obvious, lose their stainability to a considerable degree, frequently appear smaller and sometimes escape observation entirely. Moreover, the spindle fibers are no longer uniformly attached, and there are usually no polar radiations so that in the event that other granules lie in the vicinity of the spindle pole, it is impossible to distinguish among them.

An examination of Allen's figures depicting these stages does not convince one that there is a definite morphological entity which could be construed to be the polar body previously present. Allen held that in the majority of cases, a single, distinct body is present at or near the place just previously occupied by each central body, and it seemed reasonably certain to him that the latter persists in every instance, although for the reasons mentioned above, it becomes less conspicuous and its identification more difficult. Further, Allen admitted that at telophase the proportion of cases where these bodies are recognizable is even less, and in those instances when they are purported to be present, they are considerably displaced. This displacement he felt accounted for the cases in the androcyte where the blepharoplast appears not to be located at the position which was the pole of the previous division.

In the earlier antheridial divisions Allen reported the presence of polar plates or kinetosomes at the broad poles of the spindles. These were later shown by Bowen (1927) to be derived from the plastids. It may very well be that the so-called centrosome in the later androcyte mother cell divisions owes its origin to this material, but, while Allen had noted the progressive diminution of this material in successive cells and referred to its presence in the

androcyte (fig. 74), he insisted that the central body is found in addition to this material and is characterized by a system of radiating fibers. It should be noted that the polar plates and the kinetosomes were reported as having fibers running from them to the nucleus in the earlier divisions. It would seem safe to assume that the presence of fibers radiating or otherwise, would be an inadequate criterion upon which to base a distinction in this instance.

Walker (1913) also reported the presence of centrosome-like bodies in the last spermatogenous division in the antheridium of *Polytrichum* and noted a few faint astral rays. These bodies, she claimed, originate on either side of the nucleus and apparently arise de novo. She further reported that as division progresses, these bodies become less conspicuous but remain and lie close to each nucleus of the resulting androcytes. Two unusual antheridia were described, wherein an extra division was supposed to have occurred, in which the blepharoplast divided belatedly. This division occurred after nuclear division had started and after the spindle had formed. In one instance these bodies are reported as finally lying at the poles, but Walker's fig. 25 shows one at the pole and the other located halfway up the spindle. In the other instance they lie away from the pole in other parts of the cell (fig. 26). What these observations mean is difficult to say. Both Allen (1912) and Walker (1913) have cited J. and W. van Leeuwen-Reijnvaan as reporting the centrosome present in all antheridial divisions, but neither of them could confirm this report. In other mosses the centrosome has been reported as being absent (Ikeno, 1904), present only in the androcyte (Wilson, 1911; Woodburn, 1915) or in the androcyte mother cell (Bowen, 1927).

Allen (1912) reported two identifiable granules at varying distances apart, in a series of androcyte mother cells. It is worthy of note that he was able to observe this in a sufficient number of cases to convince him that they originate from a single body in the androcyte mother cell. It must be taken into consideration, however, that in all instances where some continuous action was reported, this action was not observed in a living cell but instead inferred from the appearance of a succession of fixed cells which show a granule first in one form, then in another, implying division, and then two granules seen at varying distances apart. It should

be kept in mind that the process described is based on evidence which involved neither the same body nor the same cell but presumably similar granules in successively different stages in different cells. Mottier (1904) pointed out long ago that the fact that two bodies look alike and stain alike in different cell generations is not conclusive proof that they are the same.

Taking all these discrepancies into consideration, it appears that the evidence presented for the presence of a centrosome and the centrosome-blepharoplast homology in the mosses is inadequate.

In describing the origin of the blepharoplast, the statement is often made that it first appears in the last mitosis where it functions as a centrosome. When the fact are examined, it is found that in the last mitosis a body appears at or near each of the spindle poles. This body arises de novo and not as a result of the division of previously formed bodies. To all intents and purposes it does not function at all; it is merely located at or near the spindle pole, and, since it obviously has nothing to do with the formation of the spindle or the nuclear division, it exercises no apparent function. Although the various characteristics attributed to the so-called "true" centrosomes have suffered considerable modification since the time of their first discovery, there has been no retreat from the essential concept of a centrosome as an organ which functions as a spindle-organizer and a self-propagating body (Darlington, 1949). Although the work of Yatsu (1905) and others has indicated that cytasters may be produced by artificial means, it has not been proved that, under natural conditions, true centrosomes ever originate de novo.

The fact that the blepharoplast often divides into several granules suggested to some authors (Yuasa, 1939; Sharp, 1912, 1914, 1920) that the blepharoplast still retains the power of division which characterizes the centrosome or more precisely the centriole. To the present writer there appears to be little basis in fact for this statement, since the division is regular and precise where undoubted centrosomes are found. The instances of fragmentation of the blepharoplasts that have so far been brought to light have shown no regularity of division, nor does there appear to be any semblance of precision. As described, the fragmentation occurs as a complete falling apart of the blepharoplast into a number of granules, to which it is not possible to assign any constant

number or any regularity of morphological form. The speculation that fragmentation of the blepharoplasts indicates persistence of a residual power to divide, which was characteristic of the centrosomal progenitor, is extravagant, to say the least. In most instances a distinctly uncentrosomal aspect is indicated in the subsequent coalescence of these granules. Certainly there is nothing in this phenomenon which argues for the centrosomal character of the blepharoplast, since nowhere do we find centrosomes involved in a fusion process.

It should be further pointed out that in *Marsilea,* Sharp (1914) indicated that the blepharoplast breaks up, coalesces and forms the blepharoplastic band while still in the cytoplasm, whereas Lang (1936) felt that the blepharoplast of this plant is intranuclear from a very early stage in the androcyte. It might even be argued that the body which does appear at or near the spindle pole is not necessarily the same body which is later discovered in the androcyte, since in most instances it was only conjecture that the latter is located at the point which the spindle pole was previously.

The theory that the appearance of a body at the spindle pole argues for its centrosomal origin may be questioned when this body has no discernible function here. It is true that in *Equisetum* and in *Marsilea,* Sharp brought forth evidence that the centrosome-like body is concerned with spindle formation, but this evidence suggests very serious inadequacies, as the present writer has indicated. It seems clear, then, that nowhere in the higher plants has it been conclusively proven that there is a relationship between the blepharoplast and a body having any fundamental characteristics of the centrosome. The appearance of the blepharoplast at the spindle pole seems to have no more significance than the appearance of any granule at or near this point.

Belajeff, the first to propose the centrosome-blepharoplast homology, based his proposal upon the apparent similarity of the flagella formation in *Equisetum* and the locomotor filament of the sperm of the animal. He thereby assumed that, since the blepharoplast gives rise to the flagellum in the plant and that the centrosome in the animal accomplishes essentially the same sort of thing, these organs are homologous. Bowen (1927) stated, a priori, that if a centriole-like body in plant sperms should be related to vibratile filaments, the conclusion that this body is a true centrosome would

be unavoidable. Thus upon subsequently observing the blepharoplast extending into a filament, first single, then multiple, he felt no doubt that the body to which the filaments are attached is a true centriole comparable in every way to the central bodies in animal cells. Although no one would doubt that these observations furnish some basis for comparison, it is difficult to accept the statement that the centriole of the animal cell and the blepharoplast of the plant androcyte are comparable in every way. What is more, the fact that Bowen reported indications of a third filament in some of his preparations casts some doubt on the quality of these preparations. Many others were prone to reason along these same lines, as indicated by the citation of several works by Wilson (1928) who purported to show that there are lower forms in which distinctly recognizable centrosomes are involved in the production of the locomotor apparatus, sometimes while still lying at the spindle poles. While there may be some question in these cases as to whether the proper interpretation had been placed upon these facts, this evidence lends cogency to the argument. The writer is reluctant to group all instances of the production of a locomotor apparatus in a single category, and the more recent works of Lang (1936), Yuasa (1939) and others indicate that the metamorphosis of the blepharoplast in the higher plants is much more complicated than was formerly thought. That these various structures are analogous is easily acceptable, but there is no reason to postulate homology.

Hughes (1952) has noted that there is a strong tendency to assume that observations made in a specially favorable material must be everywhere valid by virtue of their clarity and precision. As illustrative of this tendency he quotes Cleveland (1935) as stating that the close similarity between the behavior of centrioles in certain flagellated parasites and the centrioles in other cells leaves no room to doubt the general application of the observations on these flagellates to mitosis in both plants and animals.

More recently Cleveland (1953) has decried the use of the term "blepharoplast", stating that it is employed by some biologists, particularly protozoologists, when in reality they mean "centriole", and at other times when the organelle they have in mind is the basal granule. Furthermore, he suggests that there was never any need for this term. Perhaps this is true for the areas of proto-

zoology or even zoology as a whole, but the term " blepharoplast ", as originally coined by Webber (1897c) to designate the body which gives rise to the flagella in the developing male sex cells of higher plants, is a useful term for the botanist. Moreover, it should be used in reference to the structure which metamorphoses into the flagella and should not be considered in any way synonymous with the terms " centrosome " and " centriole ". Nor should it be considered to be phylogenetically or ontogenetically derived from the centrosome in the plant groups above the Thallophyta until it is established that the various motile filaments are essentially similar in origin, or until the blepharoplast in higher plants has been shown to have a definite demonstrable association with a body that is unmistakably a centrosome.

Webber (1901) pointed out from analogy with *Zamia* and *Ginkgo,* where the blepharoplast appeared in the central cell, that they could also be expected to appear in the central cell of *Marsilia* and other forms to which the central cell in the male gametophyte of *Zamia* is supposed to be homologous. It would follow then that blepharoplasts, not centrosomes, may occur in all of the spermatogenous cells resulting from the division of the central cell, as all these cells may be considered potential sperms. If we accept Sharp's work in *Marsilea,* in which he reported bodies present in three of the four spermatogenous divisions, it would appear to substantiate this view. The fact that there is a reduction in the number of cell generations between the central cell and the cell generation in which the blepharoplast appears in the progressively more highly evolved groups would seem to further support Webber's contention. In *Nephrodium* (Yamanouchi, 1908) there are four generations, in *Salvinia* (Yasui, 1911) two, and in *Zamia* the blepharoplast appears in the central cell.

It is important at this point to consider briefly the conditions which may have resulted in the loss of the blepharoplast somewhere among the progenitors of the seed plants. It seems reasonable to assume that the development of the pollen tube, which is correlated with the loss of flagellated sperms, led to the loss of the blepharoplast. Among the Cycadales and Ginkgoales the presence of the blepharoplast and motile sperms within the pollen tube represents a primitive condition.

Fragmentation of the blepharoplast and subsequent coalescence

of the fragments into a cilium- or flagellum-bearing band seem to be restricted to the higher groups and represent, perhaps, a more highly evolved condition. Sharp (1920) has reported this phenomenon in the liverwort *Blasia,* but this is contrary to Woodburn's paper (1915) on the same organism.

Conclusions

The blepharoplast ordinarily arises in the cytoplasm of the androcyte, and its origin is correlated with the developmental changes leading to the production of the mature sperm. In those instances where the blepharoplast has been reported to appear in cell generations just prior to the androcyte, its development is held to be precocious. The fact that the blepharoplast has frequently been found in the vicinity of the spindle pole or in the region which the spindle pole previously occupied is postulated to be the result of the limited space available in which it could occur or possibly as a result of the orientation of the molecules forming the spindle.

The ontogenetic origin of the blepharoplast is de novo. The phylogenetic origin is obscure, and nothing so far reported tends to shed much light upon this problem. As has been pointed out, the blepharoplast has shown no centrosomal characteristic except its reported appearance at or near the spindle pole where it performs no function. The cases of *Equisetum* and *Marsilia* are not considered to be definitive. Even though these cases were accepted as valid, it does not seem reasonable that after hundreds of mitoses without a centrosome, a centrosome should suddenly appear and carry out a spindle-forming function, even in an abortive manner.

There has been reported no evidence which might help to explain the loss of the centrosomal function, as the development of the pollen tube helps to explain the loss of the blepharoplast and attendant gametic motility in the Coniferae and the Angiospermae. The centrosome as originally described has the primary function of producing the spindle element of the mitotic figure. Nowhere in the higher plants is the evidence indisputable that bodies held to be centrosomes function in this manner. Moreover, nowhere in the plant cell does there appear to be any evidence which might be related to the probable mode of loss of the centrosomal function. The existence of the anastral spindle in the higher plants may be

explained on the basis that the spindle-forming function lies in the centromere. Pollister's theory (1945) that the centrosomes of certain snails are merely disembodied centromeres, supports this idea.

On the evidence presently available, it would seem reasonable to postulate that the centrosome was lost completely by the progenitors of the several groups of land plants. It appears highly improbable that conclusive evidence can be found in plants now extant, as to the reasons for and the manner of its disappearance.

The blepharoplast-centrosome homology as it applies to higher plants should no longer be given consideration as a valid theory, until more definitive evidence than is presently at hand can be presented for it.

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